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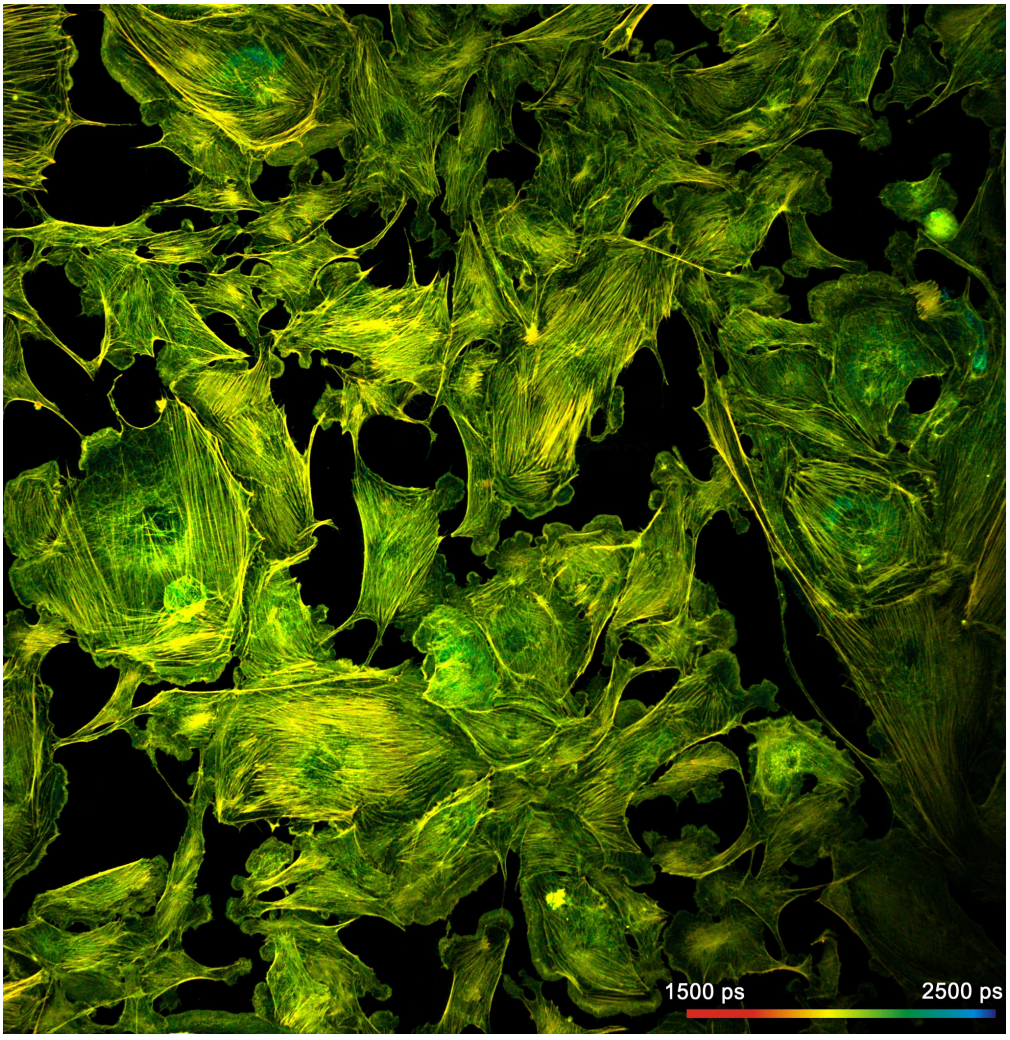
FLIM Systems for

Laser Scanning Microscopes



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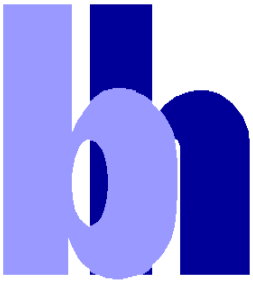

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FLIM Systems for Laser Scanning Microscopes

Overview

General Features

bh FLIM systems are unsurpassed in time resolution. With their fast detectors and negligible timing jitter of the electronics, they accurately record fluorescence-decay components which previously were unknown to even exist. Moreover, the systems feature unbelievably high timing stability. The time resolution does not degrade over extended acquisition times, see Fig. 1. Extremely weak signals or signals from extremely fragile samples can therefore be recorded successfully. The high stability in combination with sophisticated data analysis makes it unnecessary to re-calibrate the system by repeated recording of the instrument-response function (IRF). This is a significant advantage for practical use.

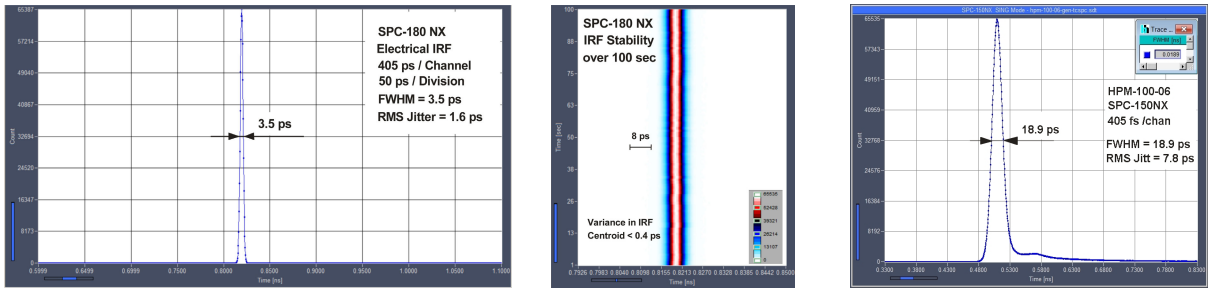


Fig. 1: Left to right: Electrical IRF of a bh FLIM system, timing stability over 100 seconds, IRF with a HPM-100-06 detector

In addition, the bh FLIM systems feature near-ideal photon efficiency and minimum acquisition time to reach a given accuracy for a given photon rate. The pixels of the recorded FLIM images contain precision fluorescence decay curves in a large number of time channels, allowing the user to derive multi-exponential decay parameters from the data. The most intriguing feature is the multi-dimensional nature of the recording process. bh FLIM systems are able to record at several excitation wavelengths simultaneously, record dynamic processes in live samples down to the millisecond range, record FLIM and PLIM simultaneously, or record multi-spectral FLIM images. With these capabilities, bh FLIM systems are able to observe several parameters of biological system simultaneously, and in their mutual dependence.

Principle

The FLIM systems are based on bh's multi-dimensional time-correlated single photon counting (TCSPC) process in combination with confocal or multiphoton scanning by a high-frequency pulsed laser beam. Each photon is characterised by its time in the laser pulse period and the coordinates of the laser spot in the scanning area in the moment of its detection. The recording process builds up a photon distribution over these parameters [24]. The result is an array of pixels, each containing a full fluorescence decay curve in a large number of time channels [27, 33, 34].

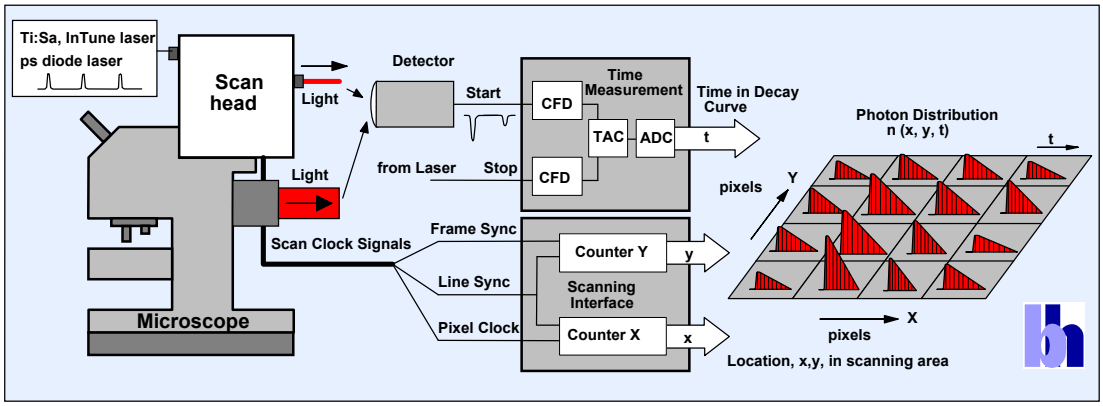


Fig. 2: bh's multi-dimensional TCSPC FLIM process probes the sample by randomly emitted photons

The principle shown in Fig. 2 works at even the fastest scan rates available in laser scanning microscopes. They combine near-ideal photon efficiency, excellent time resolution, excellent timing stability, fast recording speed, multi-wavelength capability, and resolution of multi-exponential decay functions into their components with optical sectioning capability and suppression of lateral scattering [30, 33]. The principle can be extended to record at several laser wavelengths simultaneously, record multi-wavelength FLIM images, record fast physiological effects in the sample, record spatial mosaics and Z stacks of FLIM images, or to simultaneously record fluorescence and phosphorescence lifetime images.

Most of the bh FLIM systems contain two or more of the recording channels shown in Fig. 2. By using parallel channels, high throughput is achieved, and crosstalk between the channels is avoided. The channels of a bh FLIM system can be operated with laser multiplexing to record signals excited by different laser wavelength quasi-simultaneously. The principle is shown in Fig. 3.

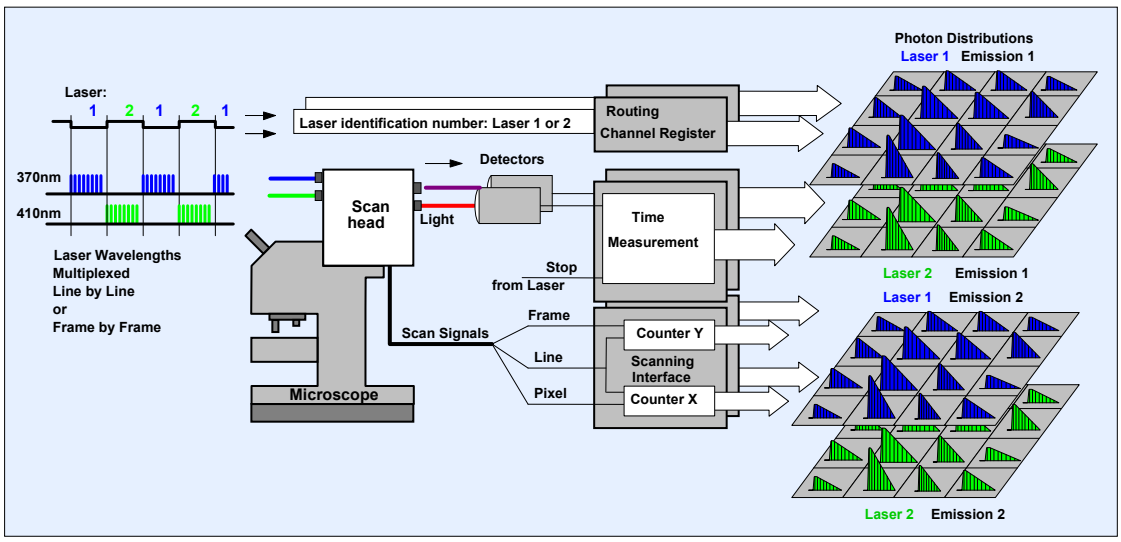


Fig. 3: Dual-channel TCSPC-FLIM system with laser multiplexing

Two lasers of different wavelength are multiplexed at high rate. The TCSPC/FLIM modules receive a signal that indicates which of the lasers was active in the moment when a photon was detected. The TCSPC modules are thus able to build up separate photon distributions for the photons excited by different lasers. With two lasers and two TCSPC modules images for four combination of excitation and emission wavelength are recorded simultaneously.

The principle shown in Fig. 2 can also be extended to simultaneously detect in 16 wavelength channels. The optical spectrum of the fluorescence light is spread over an array of 16 detector channels. The TCSPC system determines the detection times, the channel numbers in the detector array, and the position, x, and y, of the laser spot for the individual photons. These pieces of information are used to build up a photon distribution over the time of the photons in the fluorescence decay, the wavelength, and the coordinates of the image. The principle of multi-wavelength FLIM is shown in Fig. 4.

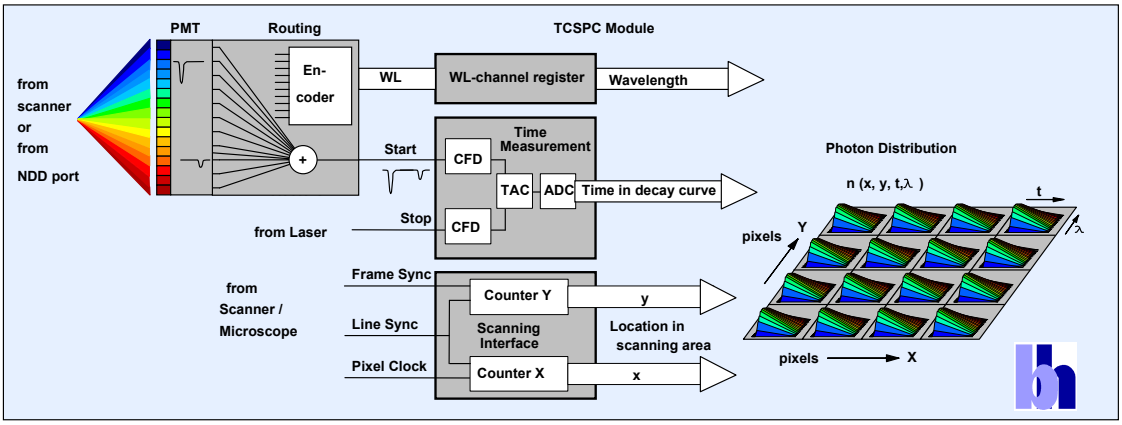


Fig. 4: Principle of Multi-Wavelength TCSPC FLIM

As for single-wavelength FLIM, the result of the recording process is an array of pixels. However, the pixels now contain several decay curves for different wavelength. Each decay curve contains a large number of time channels; the time channels contain photon numbers for consecutive times after the excitation pulse.

All bh FLIM systems are using 64-bit data acquisition software [34]. As a result, images with extremely high spatial and temporal resolution can be recorded. Images can be large as 2048 x 2048 pixels with 256 time channels per pixel, or 1024 x 1024 pixels with 1024 time channels. Such images cover the full field of view of even the best microscope lenses at diffraction-limited resolution. Multiwavelength FLIM is possible with 16 wavelength intervals and up to 512 x 512 pixels and 256 time channels.

Data Acquisition Hardware

The bh FLIM system contain one or several (usually two) TCSPC FLIM modules, a detector controller, and, if the bh DCS-120 scan head is used, a scan controller module. Different TCSPC modules, a detector controller module, and a scanner control module [24] are shown in Fig. 5.

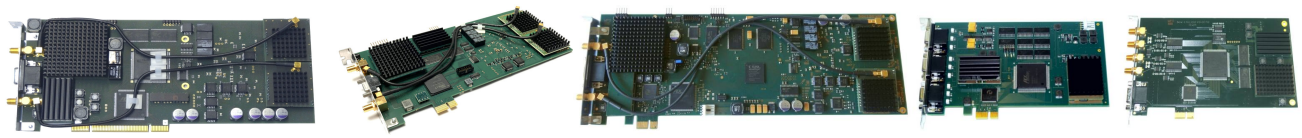


Fig. 5: Left to right: SPC-150 NX, SPC-180 NX, SPC-160 TCSPC Modules, DCC-100 detector controller, GVD-120 scan controller

The modules can be operated inside a PC, or in an extension box connected to a PC or a laptop computer, see Fig. 6.

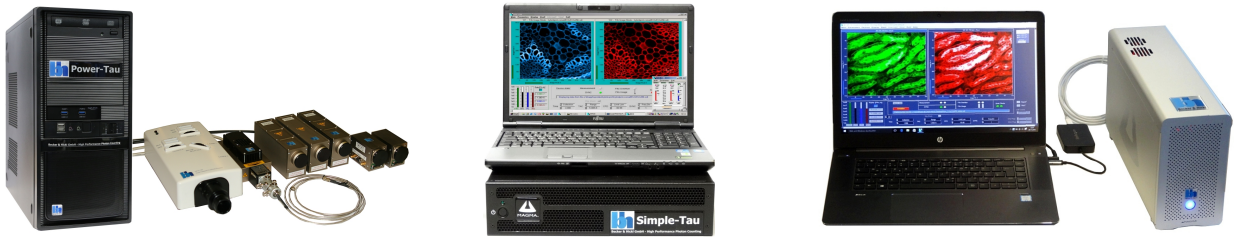


Fig. 6: Left: PC-based FLIM system, shown with DCS-120 scan head, BDL-SMC picosecond diode laser, and HPM-100 hybrid detectors. Middle: Simple-Tau 152 dual-channel FLIM system. Right: Simple-Tau II system.

Excitation Sources

bh FLIM systems are compatible with almost any high-frequency pulsed excitation source. One-photon FLIM systems usually use picosecond diode lasers, see Fig. 7.

Fig. 7: bh picosecond diode lasers. Left to right: BDS-SM laser with fibre output, BDS-SM laser with fibre coupler, LHB-104 'Laser Hub' with four lasers emitting through one single-mode fibre.

FLIM upgrades for multiphoton microscopes of external manufacturers normally use Titanium-Sapphire lasers which are integrated in these systems. The DCS-MP multiphoton system of bh is available both with a Titanium-Sapphire laser and with a single- or dual-wavelength femtosecond fibre laser [20, 34].

Detectors

Most bh FLIM systems are using the bh HPM-100 hybrid detectors [29]. The advantage of these detectors is that they have a fast and clean TCSPC response (IRF), and that they have no afterpulsing. The fast IRF and the absence of afterpulsing background have the effect that FLIM data analysis works close to the theoretical limit of photon efficiency [25]. Two versions of the HPM-100 are used for FLIM. The HPM-100-40 is used in applications which require highest sensitivity, the HPM-100-06 in applications which require highest time resolution. Detectors and detector assemblies are available with adapters for a wide variety of microscopes. The detectors for confocal ports of one-photon microscopes are compatible with those for NDD ports of multiphoton microscopes.

Fig. 8: HPM-100 hybrid detector and detector assemblies with different optical adapters

In addition to the HPMs, bh guarantee that the TCSPC systems work with any other single-photon detector as well. The systems work with single-photon avalanche diodes (SPADs), with InGaAs SPADs [3], with conventional PMTs [4], with MCP PMTs [26], and even with superconducting NbN detectors [7, 38]. Please see [34] for details.

Data Acquisition Software

The bh FLIM systems use bh SPCM data acquisition software [34]. Since 2013 the SPCM software is available in a 64-bit version. SPCM 64 bit exploits the full capability of Windows 64 bit, resulting in faster data processing, capability of recording images with extremely large pixel numbers, and availability of additional multi-dimensional FLIM modes.

The main panel of the SPCM data acquisition software is configurable by the user. Four configurations for FLIM systems are shown in Fig. 9. During the acquisition the SPCM software displays intermediate results in predefined intervals, usually every few seconds. The acquisition can be stopped after a defined acquisition time or by a user commend when the desired signal-to-noise ratio has been reached. Frequently used operation modes and user interface configurations are selected from a panel of predefined setups.

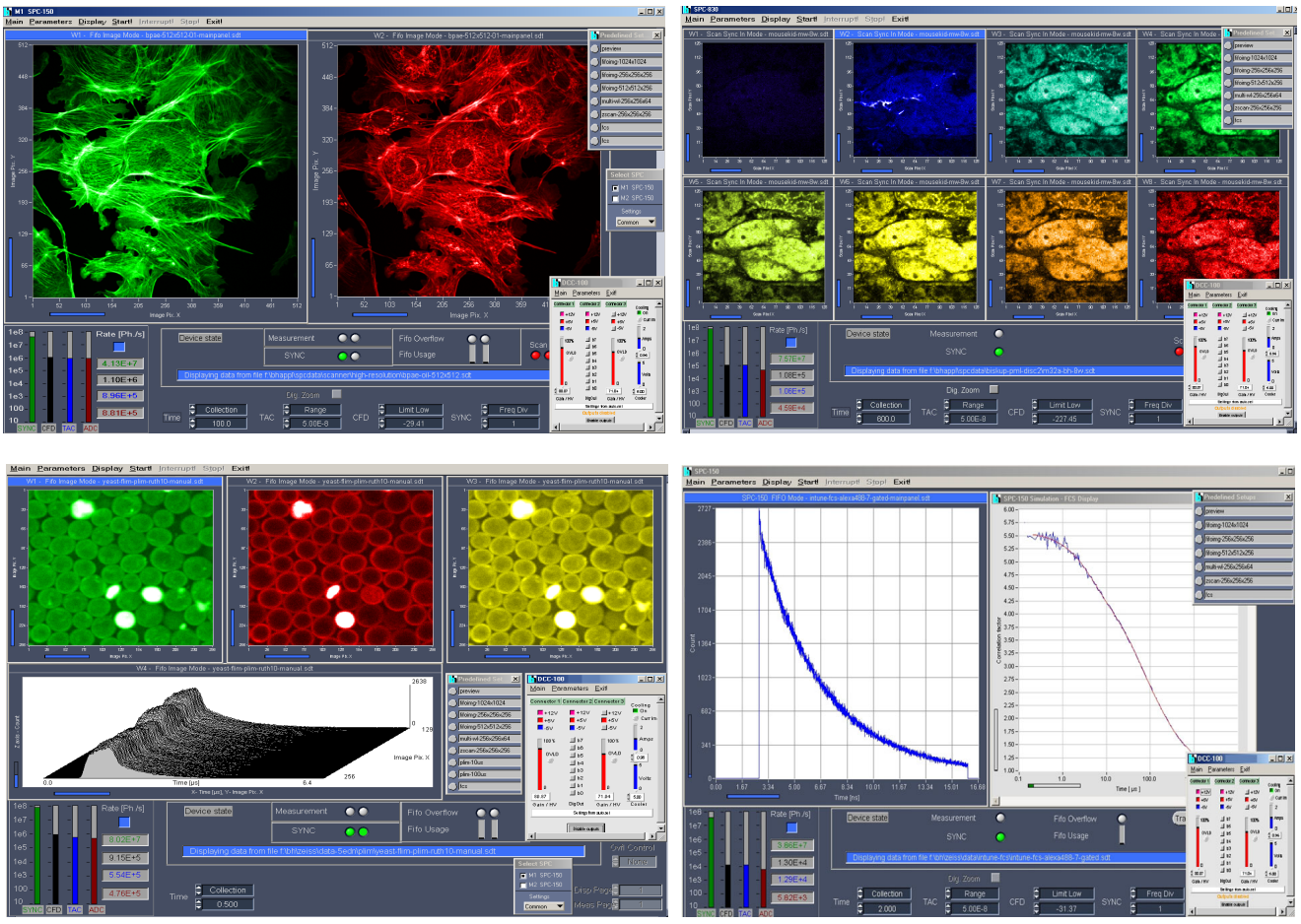


Fig. 9: SPCM software panel. Top left to bottom right: FLIM with two detector channels, multi-spectral FLIM, combined fluorescence / phosphorescence lifetime imaging (FLIM/PLIM), fluorescence correlation (FCS).

FLIM Data Analysis

All bh FLIM systems use bh SPCImage NG data analysis software. SPCImage NG runs a de-convolution on the decay data in the pixels of FLIM data. It uses single, double, or triple-exponential decay analysis to produce pseudo-colour images of lifetimes, amplitudes, or intensities of decay components, or of ratios of these parameters. An ‘incomplete decay’ model is available to determine long fluorescence lifetimes within the short pulse period of the Ti:Sa laser of a multiphoton system. Moreover, SPCImage NG avoids troublesome recording of an instrument response function (IRF) by

extracting the IRF from the FLIM data themselves. SPCImage NG uses an MLE algorithm in combination with GPU processing. This reduces the data processing time from formerly tens of minutes to a few seconds.

The main panel of the SPCImage data analysis is shown in Fig. 10. It shows a lifetime image calculated from the decay data in the pixels (left), a lifetime distribution over the pixels of a region of interest (upper middle), and the fluorescence decay curve in a selected spot of the image (lower right). The basic model parameters (one, two or three exponential components) are selected in the upper right. Please see [1, 2] or [34] for a detailed description of FLIM data analysis.

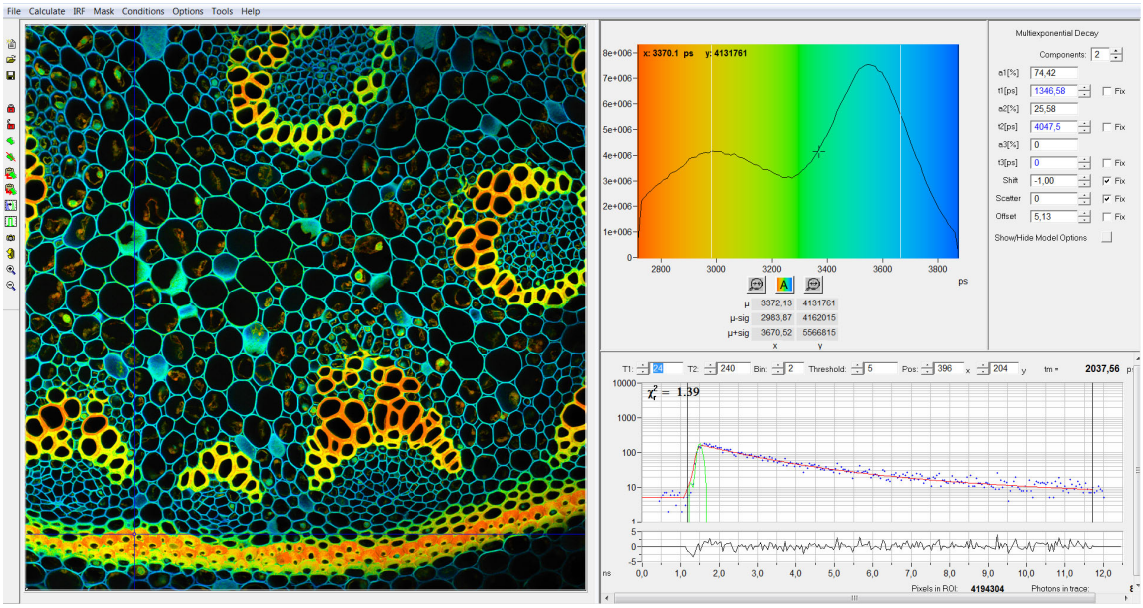


Fig. 10: Main panel of the SPCImage data analysis

Since 2018 SPCImage combines time-domain analysis with a phasor plot [40]. Pixels with different decay profiles are represented as different clusters of phasors in the phasor plot. Cells of different lifetimes therefore form separate clusters of phasors marked with different colours in the phasor plot, see Fig. 11, right.

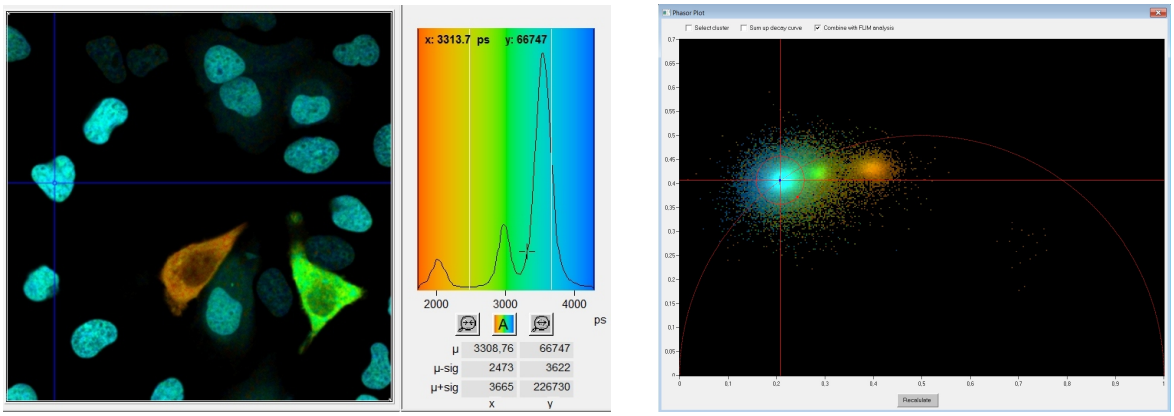


Fig. 11: SPCIMage, lifetime image and Phasor plot. The clusters in the phasor plot represent pixels of different lifetime in the lifetime image. Recorded by bh Simple-Tau 152 FLIM system with Zeiss LSM 880.

Pixels with similar phasor signature can be combined, and the combined data be used for high-accuracy multi-exponential decay analysis. Please see chapters ‘SPCImage NG Data Analysis’ in [1, 13, 34] and SPCImage NG Overview brochure [21].

FLIM Functions in Brief

Easy Change Between Instrument Configurations

Frequently used instrument configurations are stored in a 'Predefined Setup' panel. Changing between the different configurations and user interfaces is just a matter of a single mouse click, see Fig. 12.

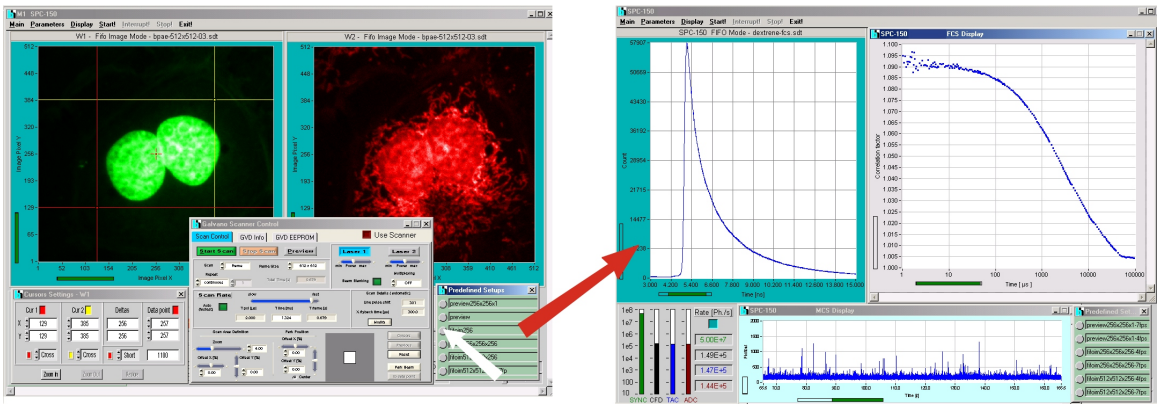


Fig. 12: Changing between different instrument configurations: The software switches from a FLIM configuration into an FCS configuration by a simple mouse click

Interactive Scan Control

Any change in the scan area of the microscope immediately becomes effective in the recorded images.

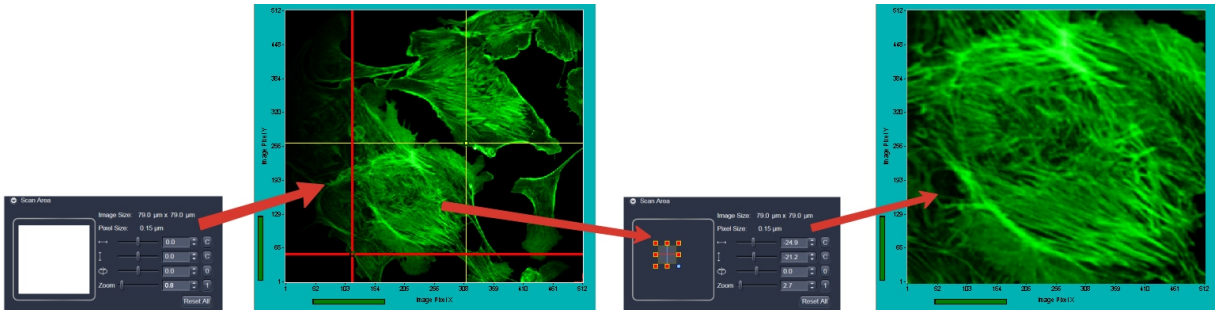


Fig. 13: Interactive scanner control for external microscope software. Example for Zeiss LSM 780/880.

For systems using the GVD-120 (such as the bh DCS 120 system) the control of the scanner is integrated in the SPCM data acquisition software. The zoom factor and the position of the scan area can be adjusted via the scanner control panel or via the cursors of the display window. Changes in the scan parameters are executed online, without stopping the scan.

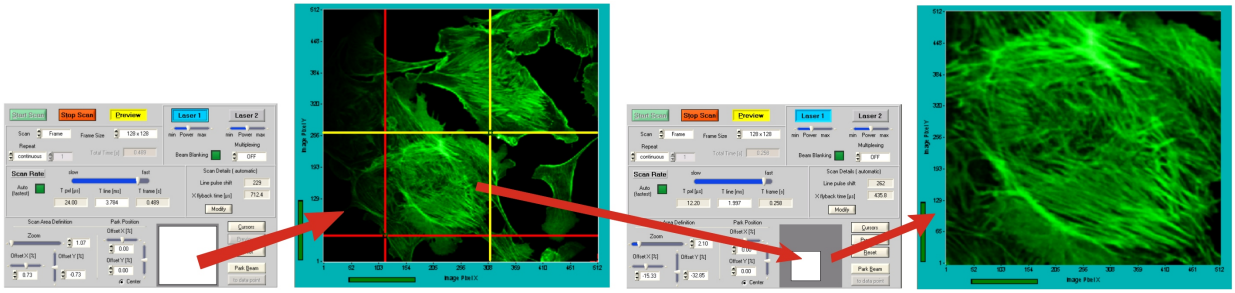


Fig. 14: Interactive scanner control for systems using the bh GVD-120 scan controller module

Fast preview function

When FLIM is applied to live samples the time and the sample exposure needed for positioning, focusing, laser power adjustment, and selection of the scan region has to minimised. Therefore, the bh FLIM systems have a fast preview function. The preview function displays images in intervals of 1 second and faster. Both intensity and lifetime images can be displayed. The preview function can be combined with fast online-FLIM display, please see Fig. 33, page 19.

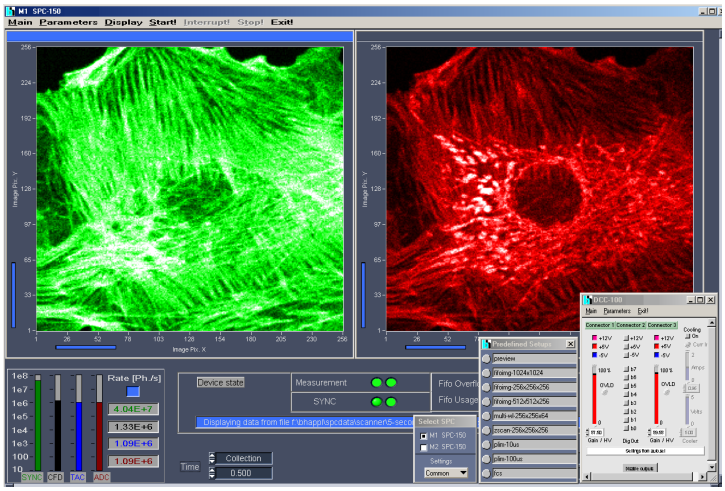


Fig. 15: SPCM software in fast preview mode. 1 image per second, two parallel FLIM channels recording in separate wavelength intervals.

Two fully parallel TCSPC FLIM Channels

Standard bh FLIM systems record in two wavelength intervals simultaneously. The signals are detected by separate detectors and processed by separate TCSPC modules [34]. There is no intensity or lifetime crosstalk. Even if one channel overloads the other channel is still able to produce correct data. More parallel channels can be added if necessary, please see [34].

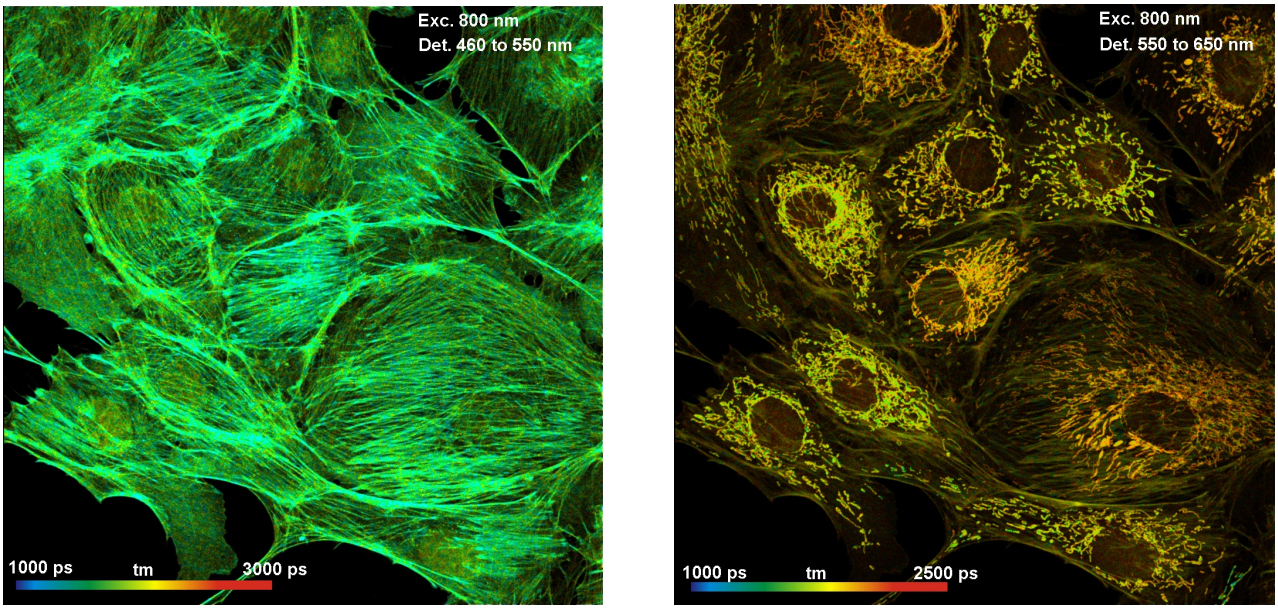


Fig. 16: Dual-channel detection. BPAE cells stained with Alexa 488 phalloidin and Mito Tracker Red. Left: 460 nm to 550 nm. Right: 550 nm to 650 nm.

Online FLIM Display

Online FLIM display is available for all versions of the bh FLIM systems. The function is based in first-moment calculation. It delivers a near-ideal signal-to-noise ratio for the single-exponential lifetime of the decay data, see [34]. An example of the SPCM main panel for dual-channel lifetime display is shown in Fig. 17.

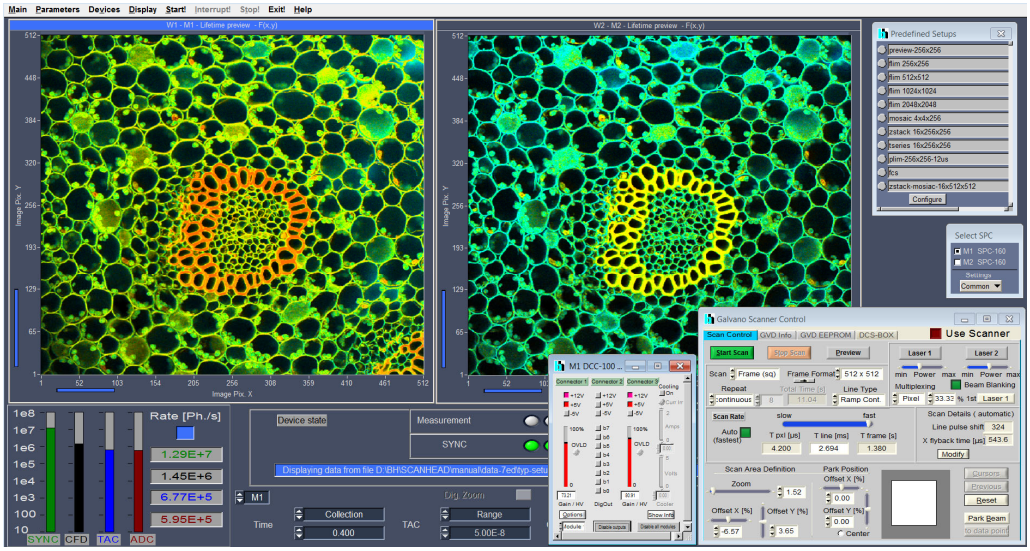


Fig. 17: SPCM main panel for online-lifetime display, dual channel system

Ultra-High Time Resolution: FLIM with <20ps IRF width

In combination with the ultra-fast HPM-100-06 and -07 detectors, bh multiphoton FLIM systems system achieve an instrument response function (IRF) of less than 20 ps FWHM [8]. The fast response greatly improves the accuracy at which fast decay components can be extracted from a multi-exponential decay. Applications are mainly in the field of metabolic imaging. In the past few years the field has been rapidly expanding [34]. Metabolic FLIM requires separation of the decay components bound and unbound NADH. Typical NADH FLIM images of the amplitude-weighted lifetime and of the amplitudes and lifetimes of the fast and slow decay component are shown in Fig. 18 and Fig. 19. Please see [10] for details. Clinical applications are described in [37, 43].

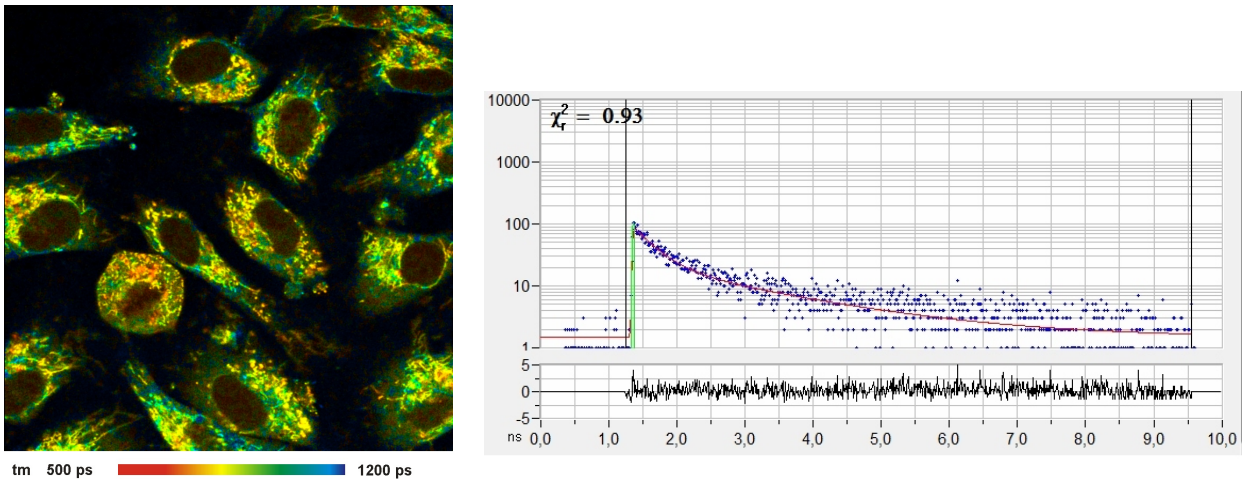


Fig. 18: NADH Lifetime image, amplitude-weighted lifetime of double-exponential fit. Right: Decay curve in selected spot, 9x9 pixel area. FLIM data format 512x512 pixels, 1024 time channels. Time-channel width 10 ps.

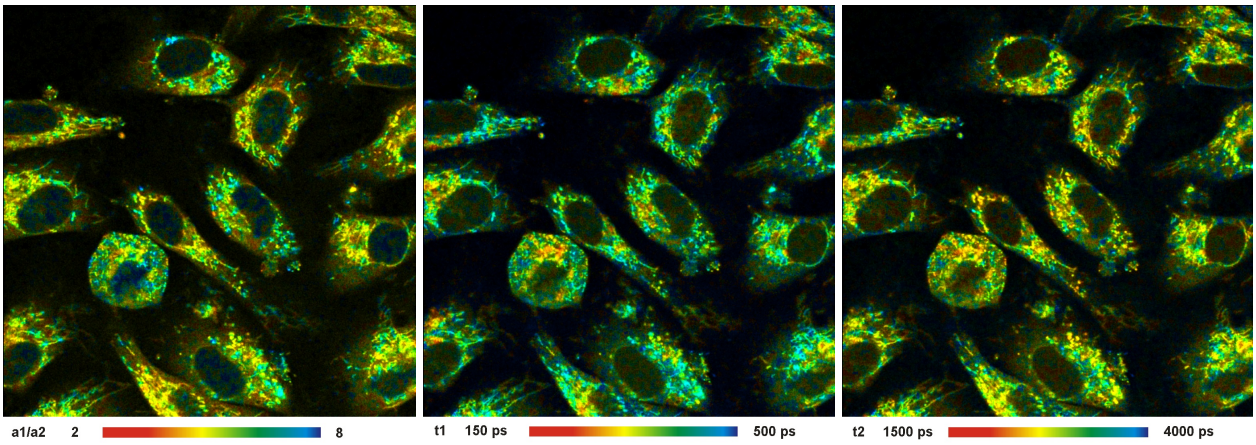


Fig. 19: Left to right: Images of the amplitude ratio, a1/a2 (unbound/bound ratio), and of the fast (t1, unbound NADH) and the slow decay component (t2, bound NADH). FLIM data format 512x512 pixels, 1024 time channels. Time-channel width 10ps.

The high time resolution of the bh multiphoton FLIM systems [20] makes fluorescence-decay components visible which have never been detected before. Fig. 20 shows FLIM data of mushroom spores, which show a dominating decay component of 12 ps lifetime [22]. In Pollen grains, the DCS-MP system detects a component with 10 ps lifetime [23], see Fig. 21. In principle, ultra-fast decay components are detectable also with other multiphoton microscopes, if the bh SPC-150 NX or SPC-180 NX TCSPC modules and HPM-100-06 detectors are used.

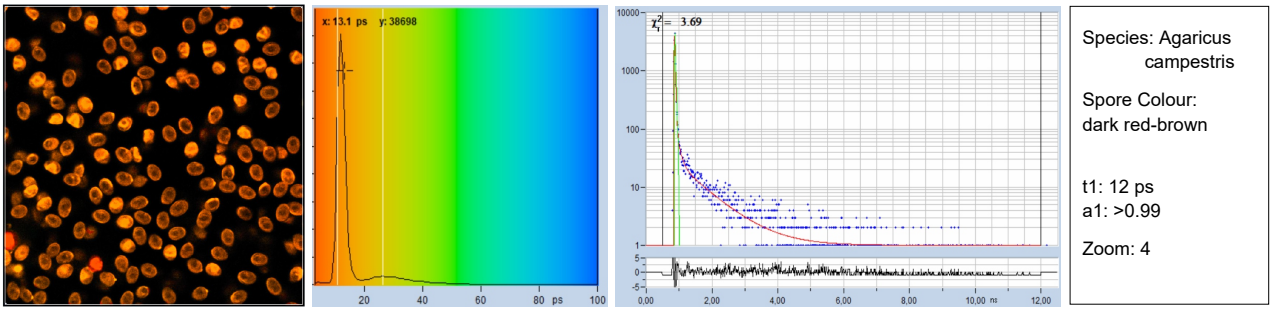


Fig. 20: 2p FLIM of Mushroom Spores. The fast component has a lifetime of t1 = 12 ps

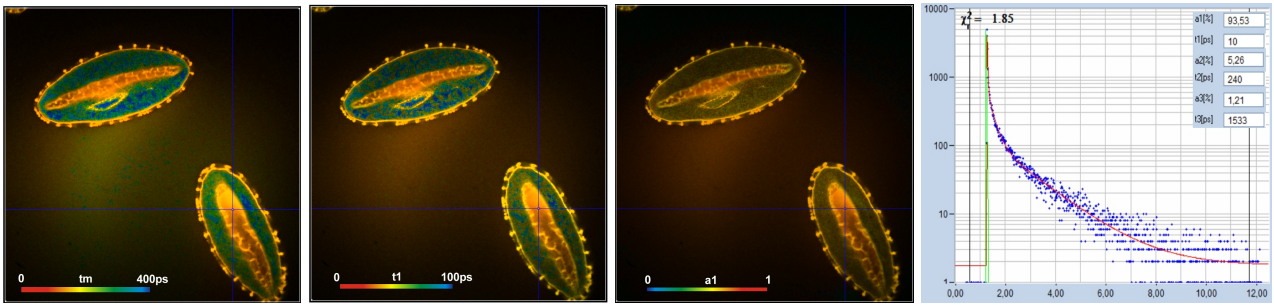


Fig. 21: 2p FLIM of Pollen Grains. The fast component has a lifetime of t1 = 10 ps

Multiphoton NDD FLIM: Clear Images from Deep Tissue Layers

bh FLIM systems for multiphoton microscopes are compatible with non-descanned detection (NDD). With non-descanned detection, fluorescence photons scattered on the way out of the sample are detected efficiently and assigned to the correct pixels of the image. The result is that bright and clear images are obtained from deep tissue layers. An example is shown in Fig. 22.

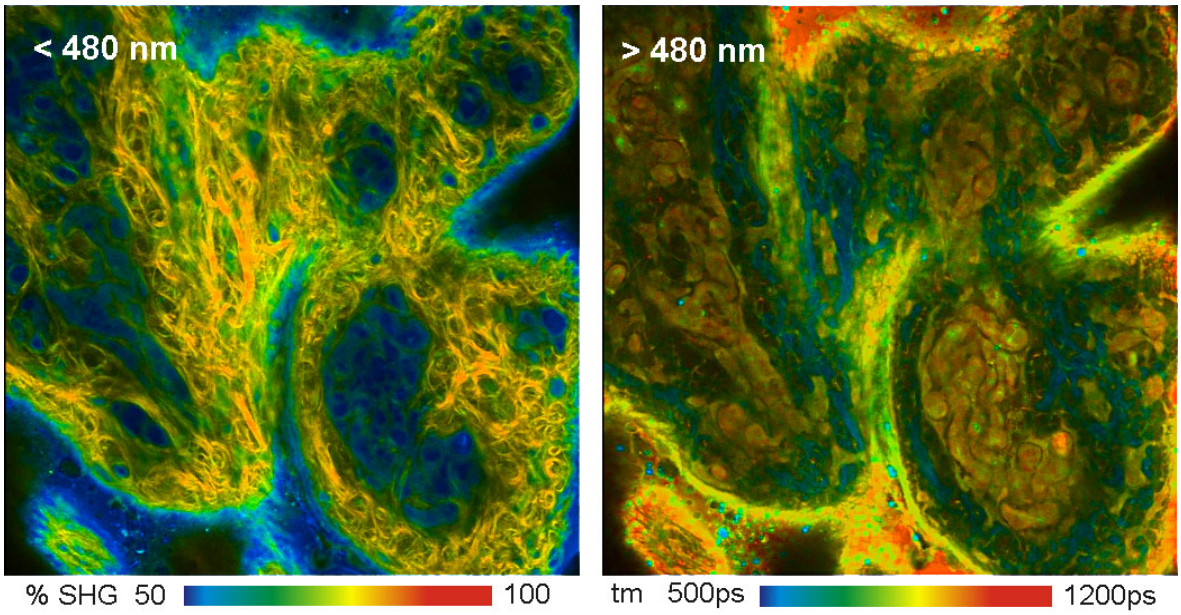


Fig. 22: Two-photon FLIM of pig skin. LSM 710 NLO, HPM-100-40, NDD. Left: Wavelength channel <480nm, colour shows percentage of SHG. Right: Wavelength channel >480nm, colour shows amplitude-weighted mean lifetime.

Metabolic FLIM by Multiplexed Excitation

The bh DCS-120 Confocal Scanning FLIM System detects changes in the metabolic state of live cells [19]. Information on the metabolic state is derived from the fluorescence decay functions of NAD(P)H and FAD. Two ps diode lasers, with wavelengths of 375nm and 405 nm, are multiplexed to alternatingly excite NAD(P)H and FAD. One FLIM channel of the DCS system detects in the emission band of NAD(P)H, the other in the emission band of FAD. A result is shown in Fig. 23.

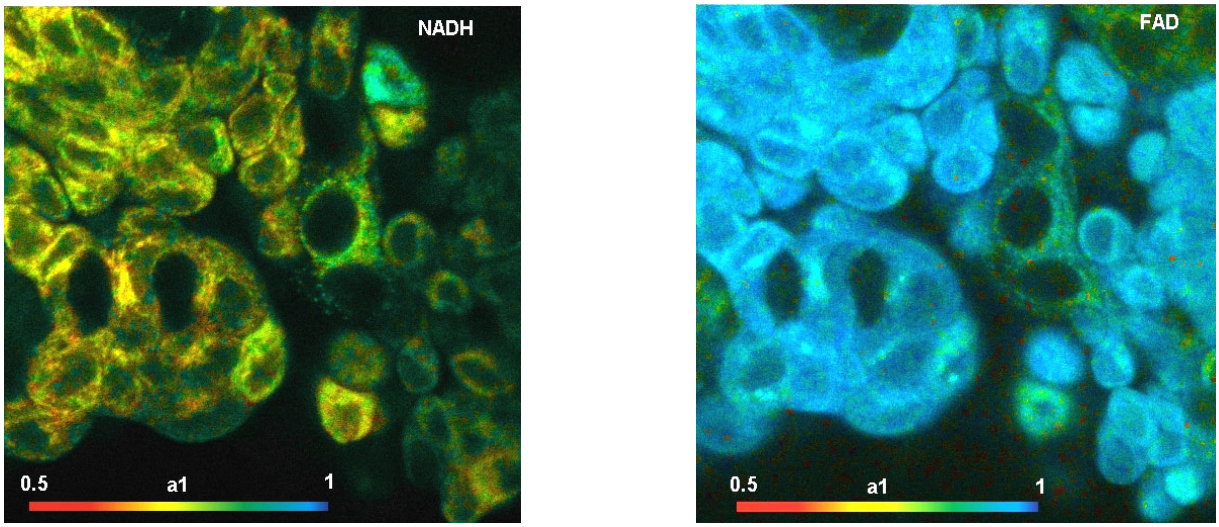


Fig. 23: a1 images (amplitude of fast component) of NAD(P)H (left) and of FAD (right)

The FLIM data are processed by SPCImage data analysis software. For both channels, the data analysis delivers images of the amplitude-weighted lifetime, tm, the component lifetimes, t1 and t2, the amplitudes of the components, a1 and a2, and the amplitude ratio, a1/a2. Moreover, it delivers the fluorescence-lifetime redox ratio (FLIRR), a_{2nadh}/a_{1fad}. For theoretical background and technical details please see [19, 34]. Clinical applications are described in [37, 43]. Metabolic FLIM can be combined with pO2 measurement by simultaneous FLIM / PLIM. Please see page 24 of this brochure.

Megapixel FLIM Images

With 64 bit SPCM software pixel numbers can be increased to 2048 x 2048 pixels, with a temporal resolution of 256 time channels. Two such images can be recorded simultaneously in different wavelength channels.

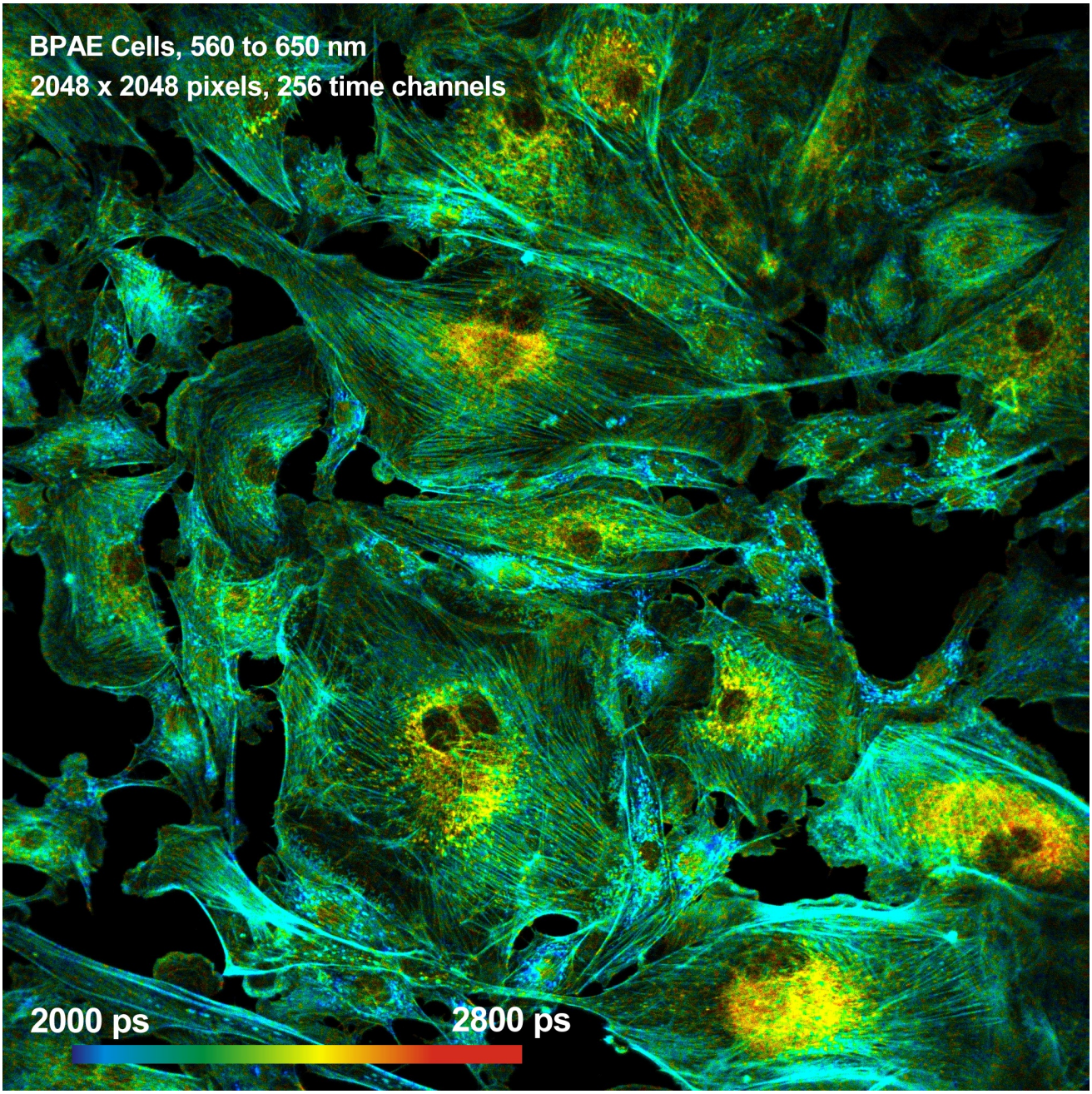


Fig. 24: BPAE cells, recorded with a spatial resolution of 2048 x 2048 pixels. 256 time channels per pixel.

With its capability to record large images the bh FLIM technique is also able to record spatial mosaic FLIM data or mosaics of images over time, depth in the sample, or emission wavelength. Please see Fig. 25, Fig. 29, Fig. 30, Fig. 36, and Fig. 37.

Multi-Spectral FLIM

bh FLIM systems are able to record simultaneously in 16 wavelength channels. The images are recorded by an extended multi-dimensional TCSPC process which uses the wavelength of the photons as a coordinate of the photon distribution [28, 34]. An example is shown in Fig. 25.

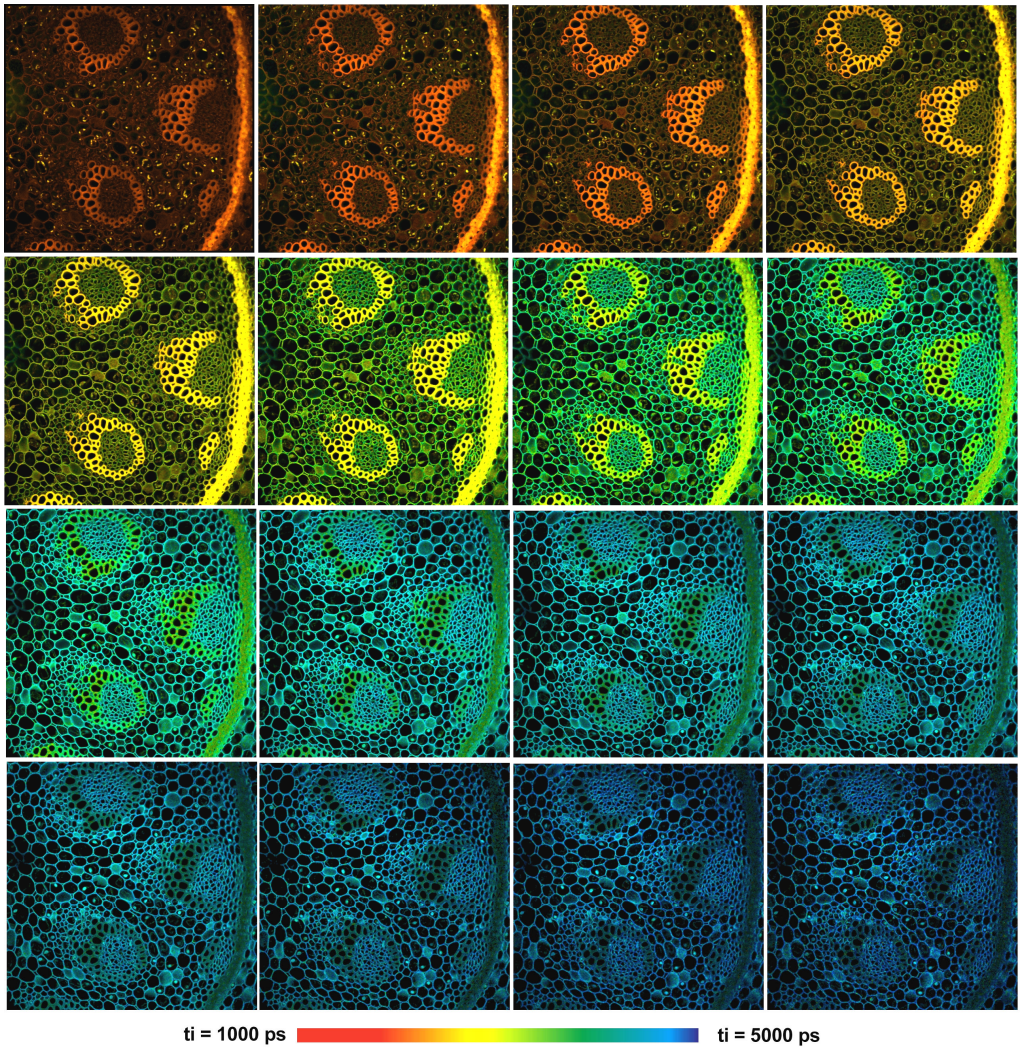


Fig. 25: Multi-wavelength FLIM, 16 images with 512 x 512 pixels and 256 time channels were recorded simultaneously. bh DCS-120 confocal scanner, bh MW-FLIM GaAsP 16-channel detector, Zeiss Axio Observer microscope.

There is no time gating, no wavelength scanning and, consequently, no loss of photons in this process. The system thus reaches near-ideal recording efficiency. Moreover, dynamic effects in the sample or photobleaching do not cause distortions in the spectra or decay functions. The individual images in the 16 wavelength channels are recorded at a resolution of up to 512x512 pixels and 256 time channels.

Fig. 26 and Fig. 27 demonstrate the true resolution of the data. Images from two wavelength channels, 502 nm and 565 nm, were selected form the data shown Fig. 25, and displayed at larger scale and with individually adjusted lifetime ranges. With 512x512 pixels and 256 time channels, the spatial and temporal resolution of the individual images is comparable with that normally used for single-wavelength FLIM.

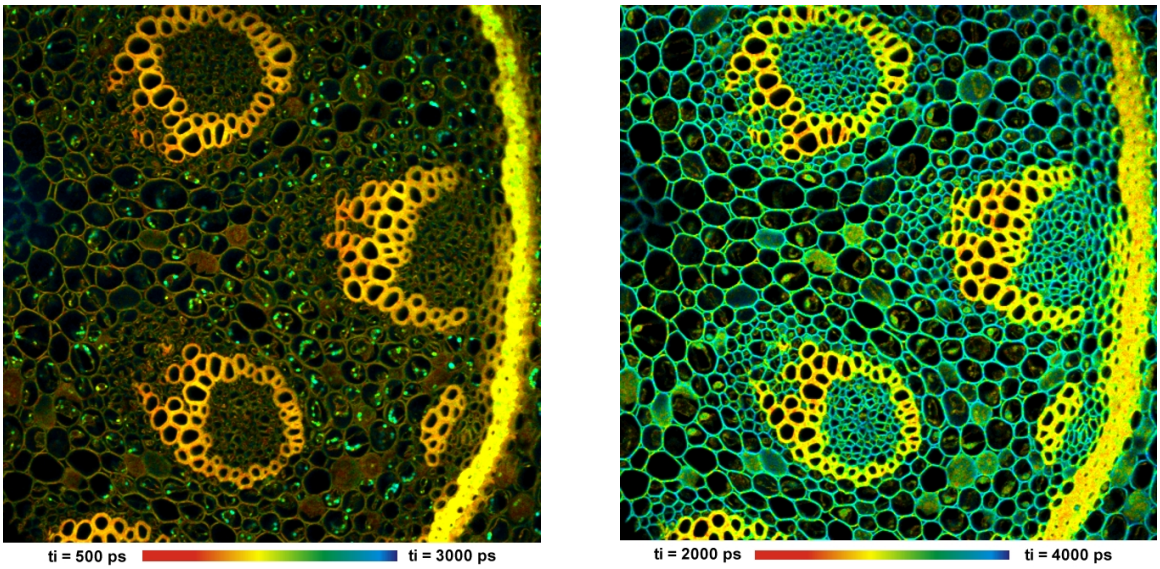


Fig. 26: Two images from the array shown in Fig. 25, displayed in larger scale and with individually adjusted lifetime range. The images have 512 x 512 pixels and 256 time channels.

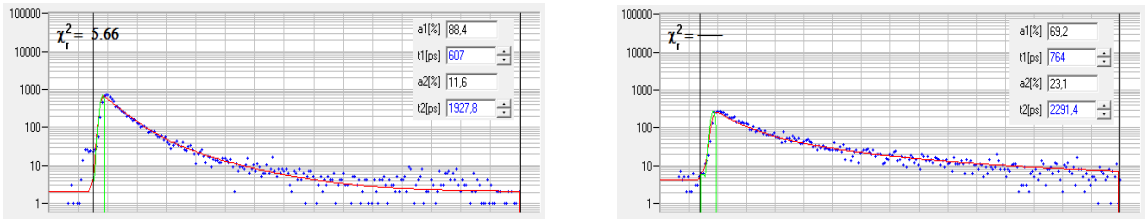


Fig. 27: Decay curves at selected pixel position in the images shown above. Blue dots: Photon numbers in the time channels. Red curve: Fit with a double-exponential model.

Multiphoton Multispectral NDD FLIM

bh's MW FLIM is the world's first simultaneously detecting multiphoton multispectral NDD FLIM system [28]. It uses a special optical interface that connects the NDD ports of multiphoton microscopes to the input slit of the detector [1, 2, 34]. A typical result is shown in Fig. 28.

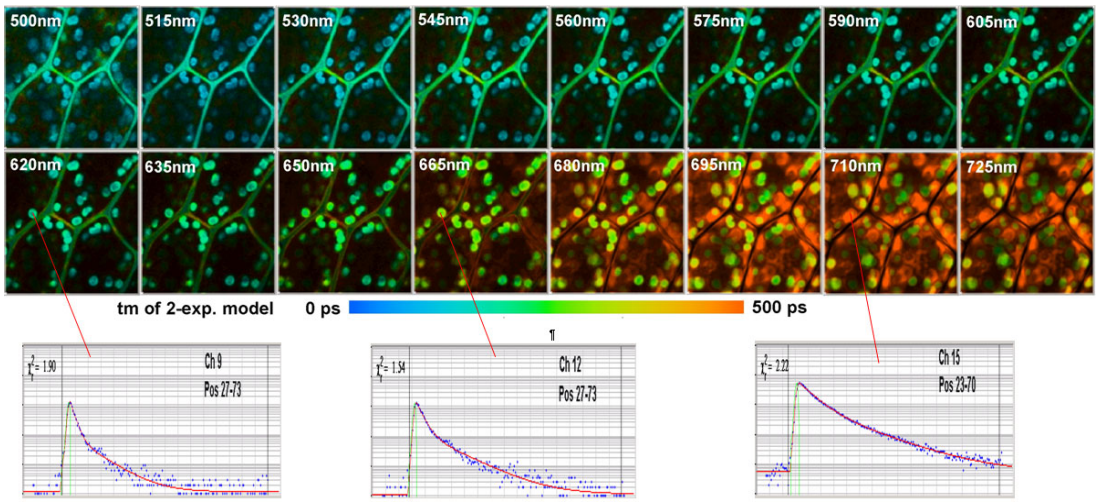


Fig. 28: Multiphoton Multispectral NDD FLIM. Plant tissue, lifetime images and decay curves in selected pixels and wavelength channels. Recorded with LSM 710 NLO and bh MW FLIM detector

Lateral Mosaic FLIM

Mosaic FLIM is based on bh's 'Megapixel FLIM' technology introduced in 2014. Mosaic FLIM records a large number of images into a single FLIM data array [34]. The individual images within this array can be for different displacement of the sample (lateral mosaic), different depth within the sample (z-stack mosaic), of for different times after a stimulation of the sample (temporal mosaic). Lateral mosaic FLIM combines favourably with the Tile Imaging capability of the Zeiss LSM 710/780/880 and similar procedures in other microscopes. An example is shown in Fig. 29. The complete data array has 2048 x 2048 pixels, and 256 time channels per pixel. Compared to a similar image taken through a low-magnification lens the advantage of mosaic FLIM is that a lens of higher numerical aperture can be used, resulting in higher detection efficiency and higher spatial resolution.

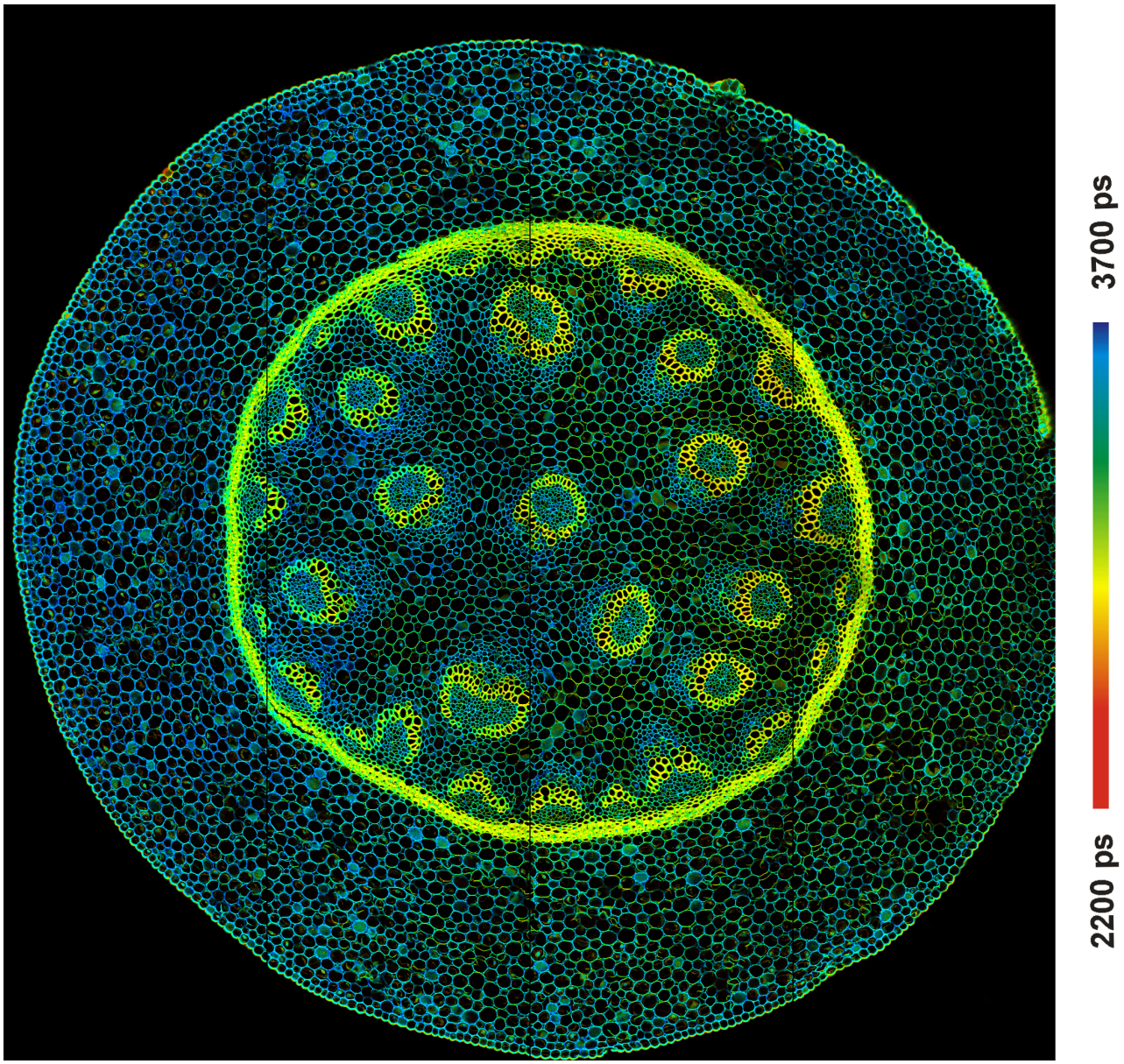


Fig. 29: Mosaic FLIM of a Convallaria sample. The mosaic has 4x4 elements, each element has 512x512 pixels with 256 time channels. The complete mosaic has 2048 x 2048 pixels, each pixel holding 256 time channels. Zeiss LSM 710 with bh Simple-Tau 150 FLIM system. Total sample size covered by the mosaic 2.5 x 2.5 mm.

Z Stack Mosaic FLIM

The Mosaic FLIM function can be used to record Z Stacks of FLIM images. As the microscope scans consecutive image planes the FLIM system records the data into consecutive elements of a FLIM mosaic. The advantage over the traditional record-and-save procedure (page 18) is that no time has to be reserved for save operations, and that the entire array can be analysed in a single data analysis run.

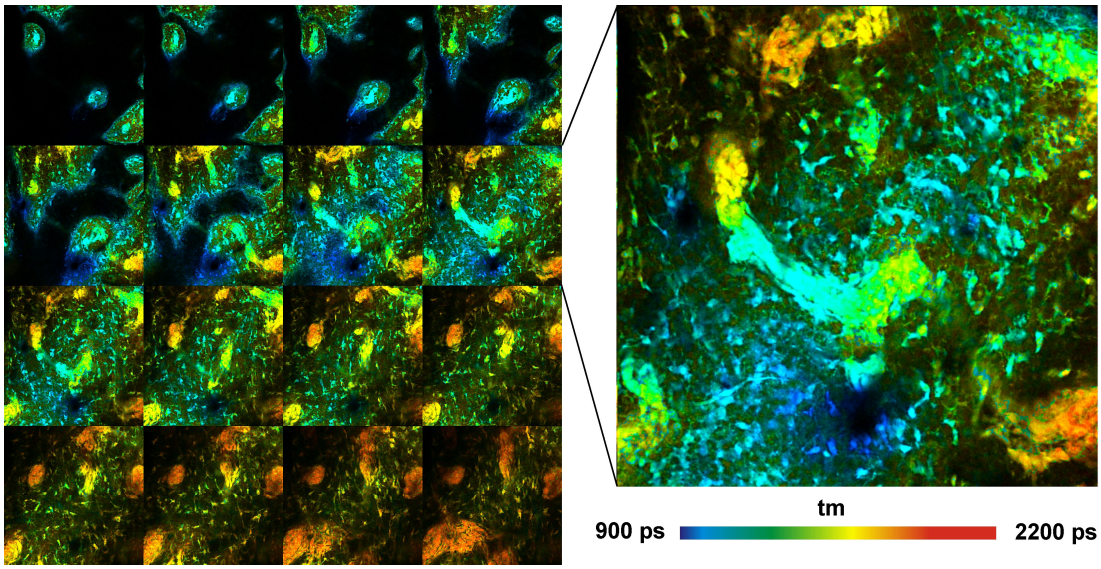


Fig. 30: FLIM Z-stack, recorded by Mosaic FLIM. Pig skin stained with DTTC. 16 planes, 0 to 60 um from top of tissue. Each element of the FLIM mosaic has 512x512 pixels and 256 time channels per pixel. Plane 8 is shown magnified on the right. LSM 7 OPO system, HPM-100-50 GaAs hybrid detector.

Z Stack Recording by Record-and-Save Procedure

The bh FLIM systems are able to record Z stacks of FLIM images [1, 34] also by a conventional record-and-save procedure. For each Z plane, a FLIM image is scanned and acquired for a specific ‘collection time’. Then the data are saved in a file, the microscope steps to the next plane, and the next image is acquired. The procedure continues for a specified number of Z planes. A Z stack of autofluorescence images taken at a water flee is shown in Fig. 31.

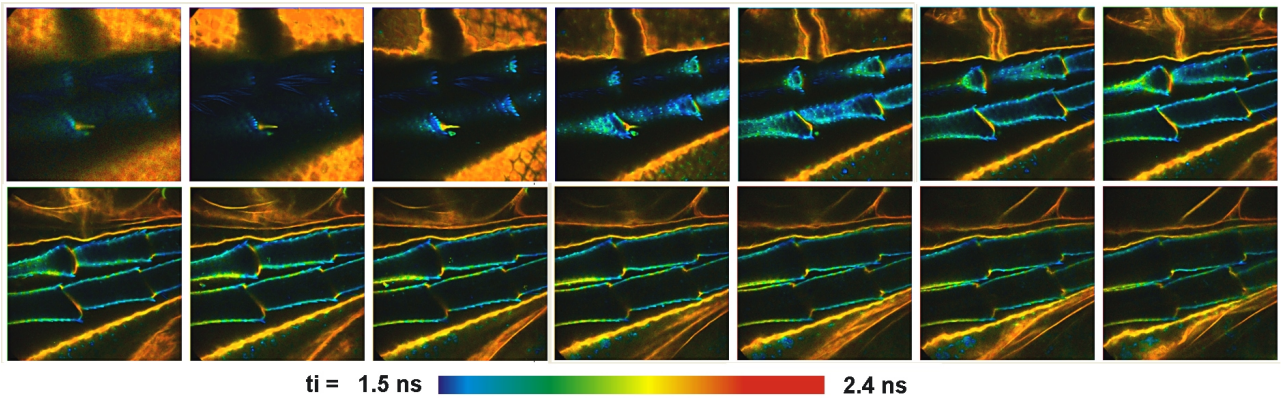


Fig. 31: Z stack recording, part of a water flee, autofluorescence. Images 256x256 pixels, 256 time channels.

Another way of recording Z stacks is by Mosaic FLIM. In that case, the images of the individual planes are recorded in subsequent elements of a FLIM data mosaic. Please see ‘Z Stack Mosaic FLIM’, page 18.

Time-Series FLIM by Record-and-Save Procedure

Time-series FLIM is available for all system versions, and all detectors [1, 2, 34]. Time series as fast as 2 images per second can be obtained. A time series taken at a moss leaf is shown in Fig. 32. Time-series FLIM at higher speed can be performed by temporal mosaic FLIM, see Fig. 36 and Fig. 37. Time-series FLIM can be combined with online-FLIM display, please see section above.

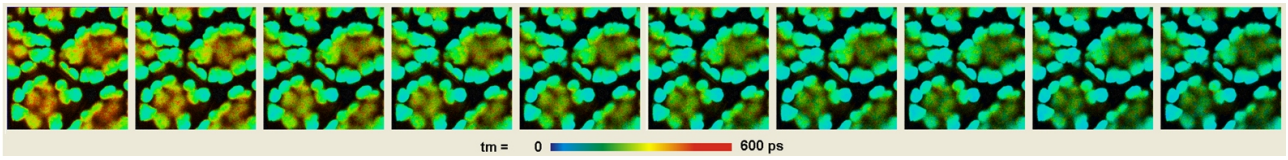


Fig. 32: Time-series FLIM, 1 image per second. Chloroplasts in a leaf, the fluorescence lifetime of the chlorophyll decreases with the time of exposure.

Fast Online FLIM

The bh TCSPC/FLIM systems record and display fluorescence lifetime images at a rate of up to 10 images per second [11, 14]. The function is normally used to select interesting cells within a larger sample for subsequent high-accuracy FLIM acquisition. In FLIM experiments with longer acquisition time it helps the user evaluate the signal-to-noise ratio of the data and decide whether enough photons have been recorded to reveal the expected lifetime effects in the sample.

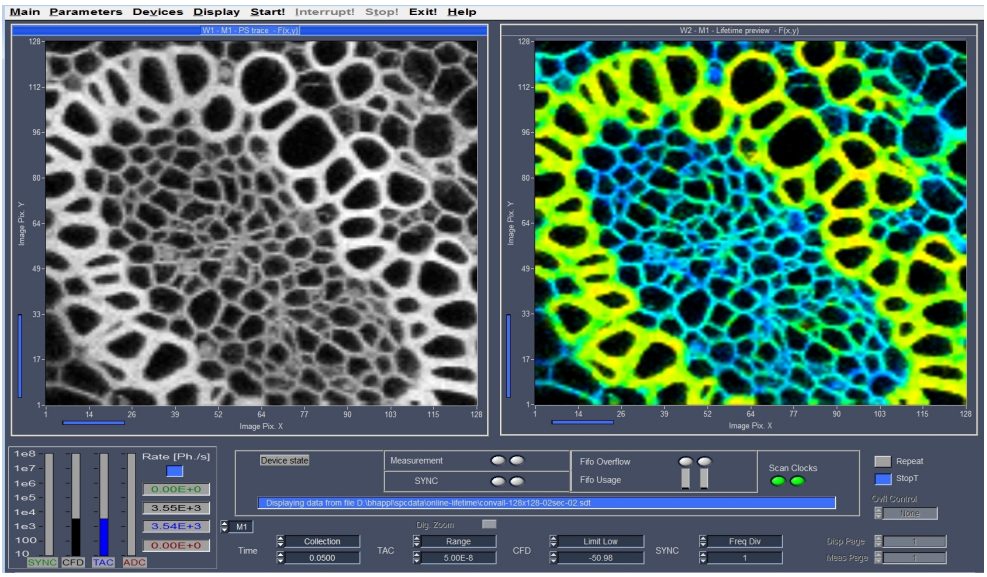


Fig. 33: Fast online FLIM. Intensity image (left) and lifetime image (right). Images 128 x 128 pixels, recorded at a speed of 5 images per second.

The bh FASTAC Fast-Acquisition FLIM System

The bh Fast-Acquisition FLIM system uses four parallel TCSPC channels and a device that distributes the photon pulses of a single detector into the four recording channels [15, 16, 17]. The system features an electrical IRF width of less than 7 ps (FWHM), and a time channel width down to 820 fs. The optical time resolution with an HPM-100-06 or -07 hybrid detector is shorter than 25 ps (FWHM). The system is virtually free of pile-up effects. FLIM data can be recorded at acquisition times down to the fastest frame times of the commonly used galvanometer scanners. The data are recorded with the TCSPC-typical number of time-channels of up to 4096, and with pixel numbers from 128 x 128 to

2048 x 2048 pixels. The system is therefore equally suitable for fast FLIM and precision FLIM applications.

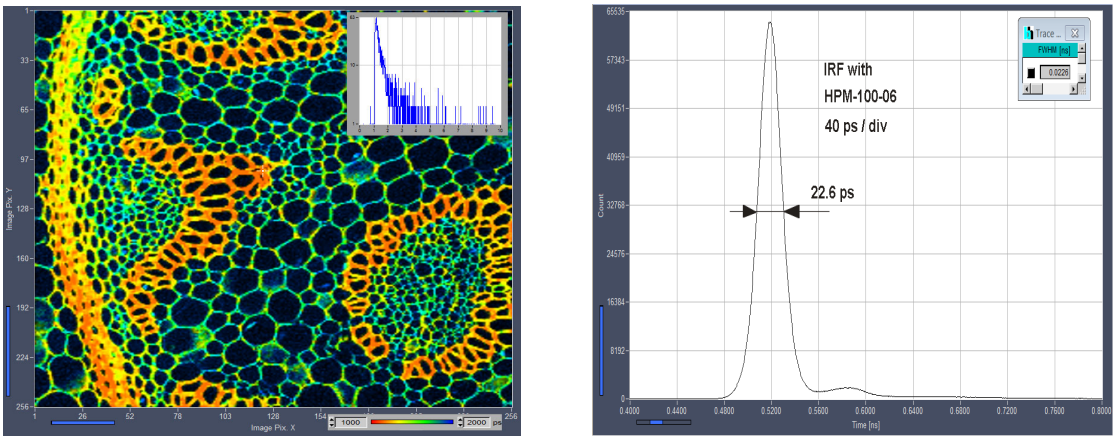


Fig. 34: FASTAC FLIM. Left: 256x256 pixels, acquisition time 0.5 s. Insert: Decay data in 10x10 pixel area. Right: IRF

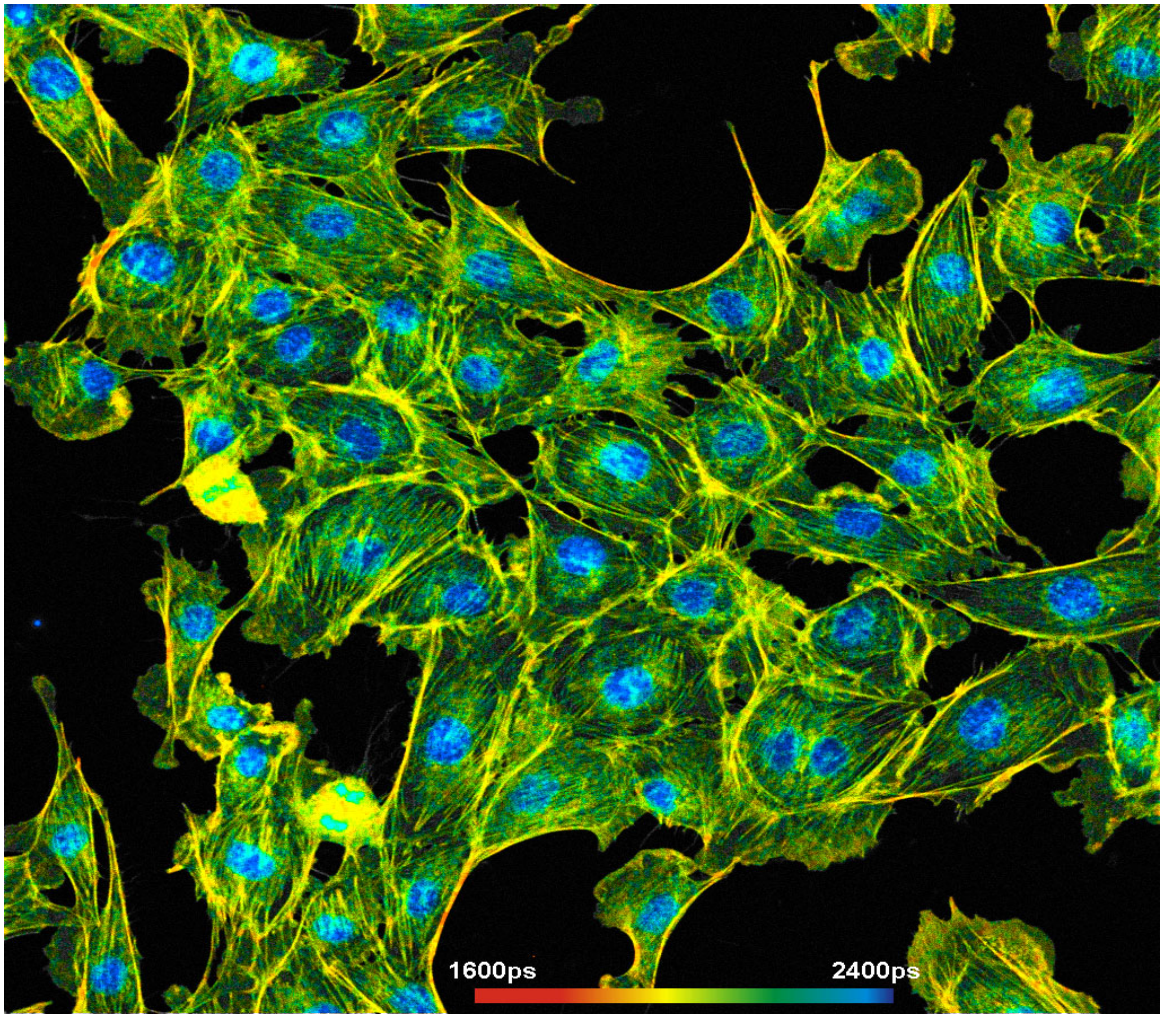


Fig. 35: High-accuracy FLIM image, recorded in 10 seconds. 1024 x 1024 pixels, 1025 time channels. FASTAC FLIM system with Zeiss LSM 880 NLO multiphoton microscope.

Time-Series Recording by Temporal Mosaic FLIM

Mosaic FLIM can be used to record FLIM time series. The recording principle is the same as for lateral mosaic FLIM, except for the fact that the sample is not moved between the individual recordings. The result is thus a mosaic of FLIM images for consecutive times after the start of an experiment. An example is shown in Fig. 36.

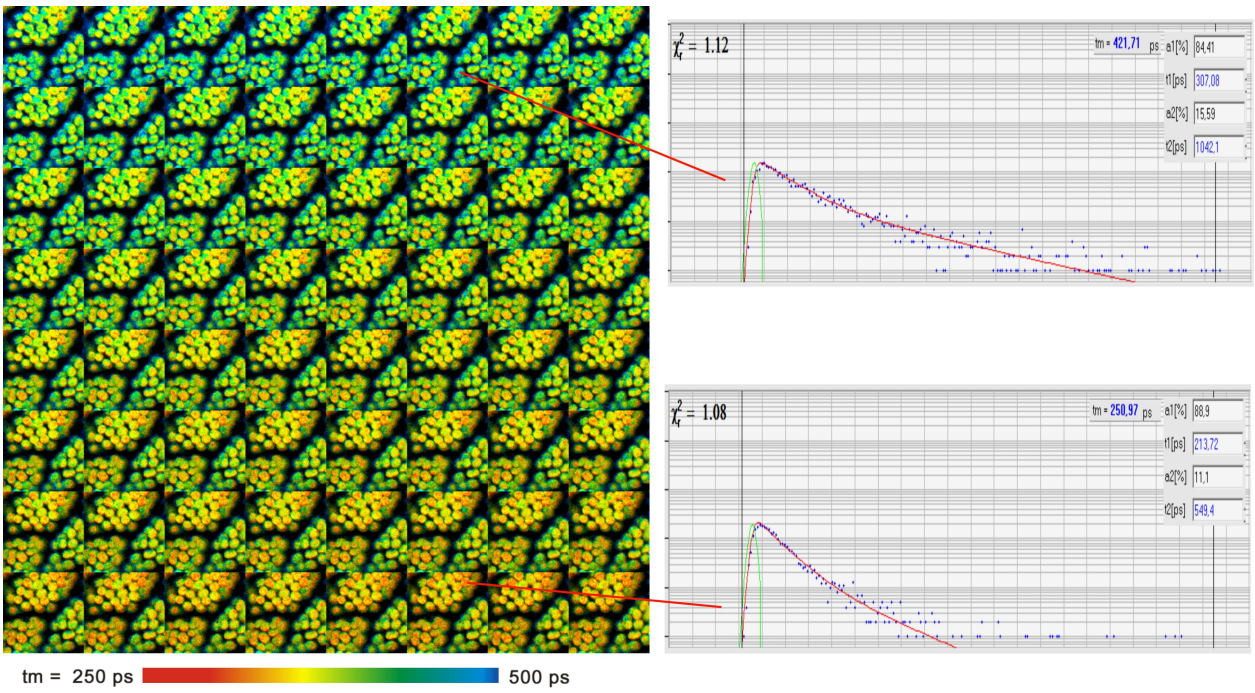


Fig. 36: Time series acquired by mosaic FLIM. Recorded at a speed of 1 mosaic element per second. 64 elements, each element 128 x 128 pixels, 256 time channels, double-exponential fit of decay data. Sequence starts at upper left. Moss leaf, lifetime changes by non-photochemical chlorophyll transient.

Faster than Fast FLIM: Temporal Mosaic FLIM with Triggered Accumulation

The advantage of Mosaic FLIM is that no time has to be reserved for save operations between the recording of the individual images. A Mosaic-FLIM time series can therefore be made very fast. The most important advantage is, however, that temporal Mosaic FLIM data can be accumulated. A lifetime change in the sample is stimulated periodically, and a mosaic recording sequence started for each stimulation. Because the entire photon distribution is kept in the memory the photons from the subsequent runs are automatically accumulated. The result is that the signal-to-noise ratio no longer depends on the speed of the series. The only speed limitation is the minimum frame time of the scanner. For many laser scanning microscopes frame times of less than 50 milliseconds can be achieved [39]. This brings the transient-time resolution down to the range where physiological effects in live samples occur. A typical application is the recording of Ca^{2+} transients in neurons. An example is shown in Fig. 37.

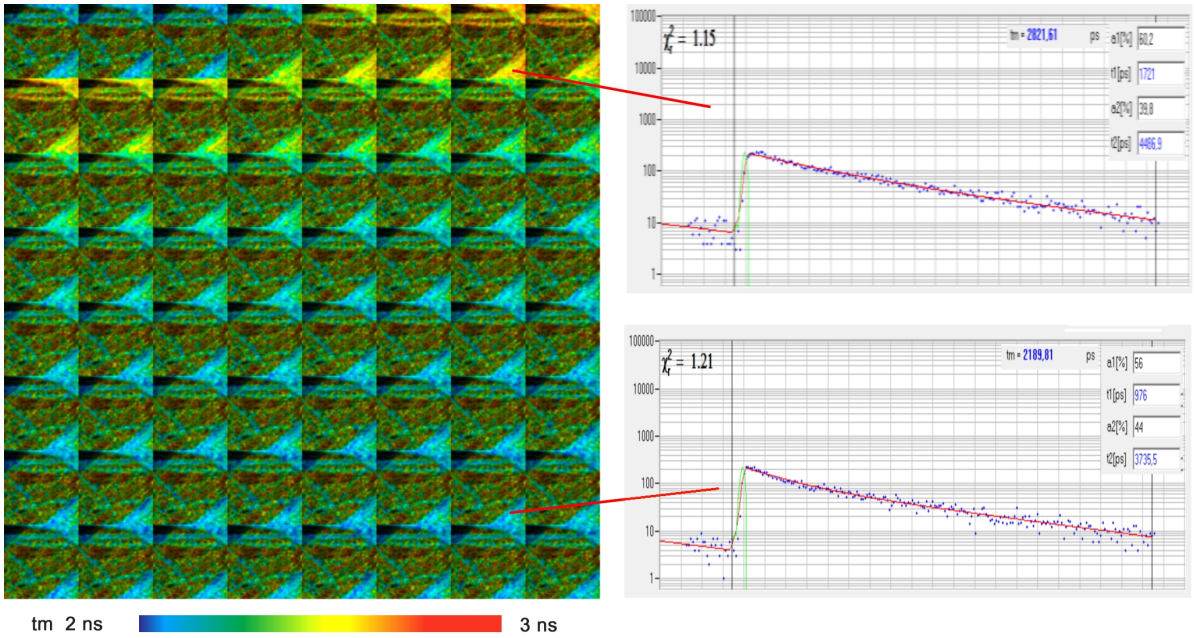


Fig. 37: Temporal mosaic FLIM of the Ca^{2+} transient in cultured neurons after stimulation with an electrical signal. The time per mosaic element is 38 milliseconds, the entire mosaic covers 2.43 seconds. Experiment time runs from upper left to lower right. Photons were accumulated over 100 stimulation periods. Zeiss LSM 7 MP multiphoton microscope and bh SPC-150 TCSPC module. Data courtesy of Inna Slutsky and Samuel Frere, Tel Aviv University, Sackler Faculty of Medicine.

FLITS: Fluorescence Lifetime-Transient Scanning

FLITS records transient effects in the fluorescence lifetime of a sample along a one-dimensional scan. The technique is based on building up a photon distribution over the distance along the scan, the arrival times of the photons after the excitation pulses, and the experiment time after a stimulation of the sample. The maximum resolution at which lifetime changes can be recorded is given by the line scan time. With repetitive stimulation and triggered accumulation transient lifetime effects can be resolved at a resolution of about one millisecond [31].

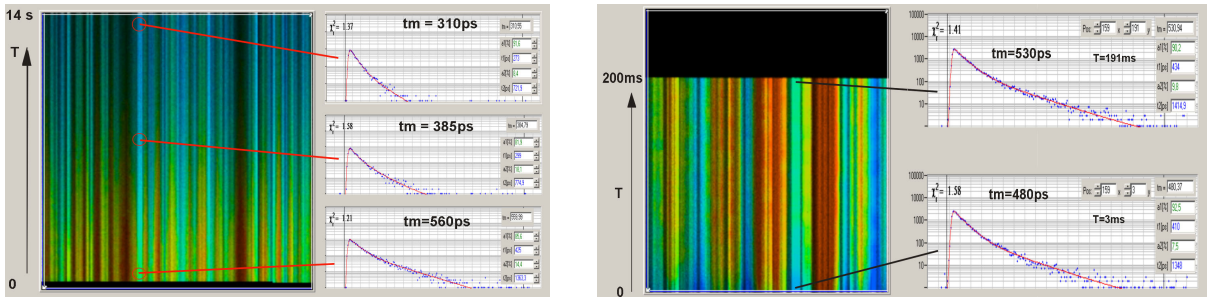


Fig. 38: FLITS of chloroplasts in a grass blade, change of fluorescence lifetime after start of illumination. Left: Non-photochemical transient, transient resolution 60 ms. Right: Photochemical transient. Triggered accumulation, transient resolution 1 ms.

Excitation Wavelength Multiplexing

By multiplexing several ps diode lasers images can be obtained quasi-simultaneously for different excitation wavelength [34]. With the two detection channels of the bh systems, images for three or four combinations of excitation and emission wavelength are obtained. An example is shown in Fig. 39.

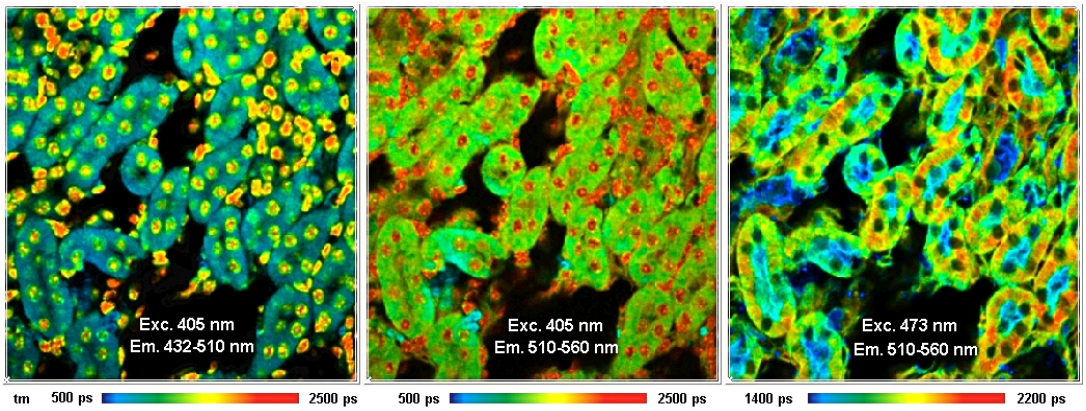


Fig. 39: Excitation wavelength multiplexing, 405 nm and 473 nm. Detection wavelength 432 nm to 510 nm and 510 nm to 550 nm. Mouse kidney section, stained with Alexa 488 WGA, Alexa 568 phalloidin, and DAPI.

Near-Infrared FLIM

Scattering coefficients in biological tissue in the near-infrared region are lower than in the visible. Therefore, FLIM with near-infrared dyes is a second way to obtain images from deep layers of biological tissues. Different than for multiphoton FLIM, where only the excitation is in the NIR, both the excitation and the emission are in the near infrared. Therefore, deep-tissue imaging is possible even with one-photon excitation and confocal detection. Moreover, many near-infrared dyes display large lifetime variations with the local molecular environment and are thus potential molecular markers. Near-infrared FLIM can be performed by one-photon excitation with ps diode lasers, by one-photon excitation with Ti:Sapphire lasers, or two-photon excitation by an OPO [5, 32]. Please see Fig. 40, Fig. 41 and Fig. 42.

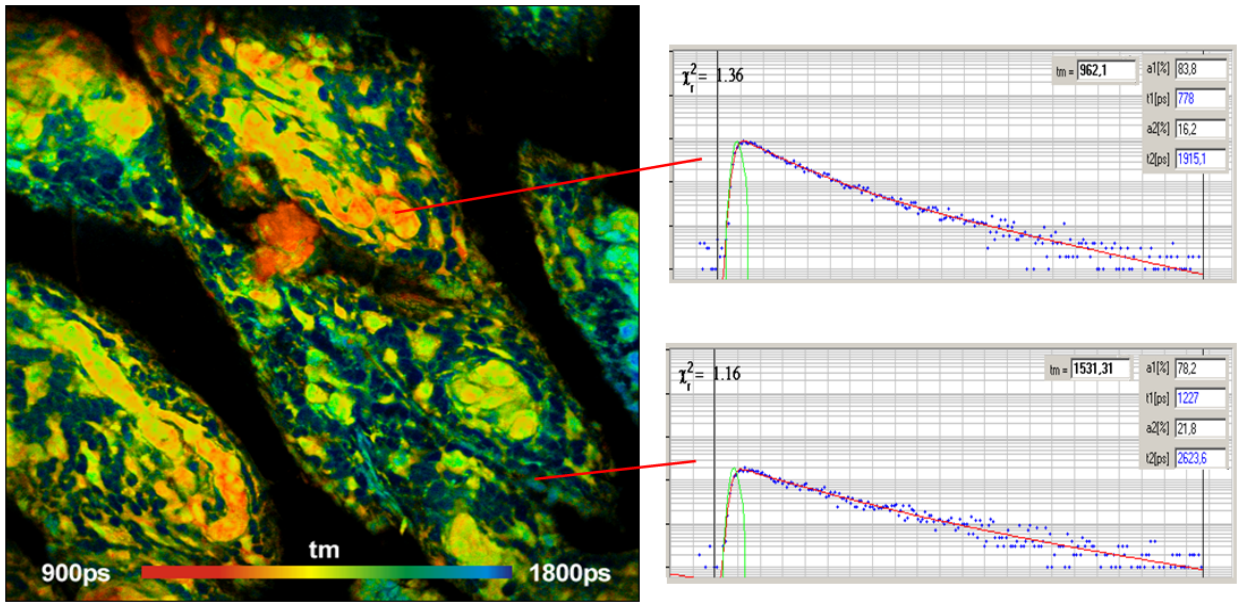


Fig. 40: Near-Infrared FLIM with picosecond diode laser, bh DCS-120 system. Pig skin sample stained with 3,3'-diethylthiatricarbocyanine, detection wavelength from 780 nm to 900 nm.

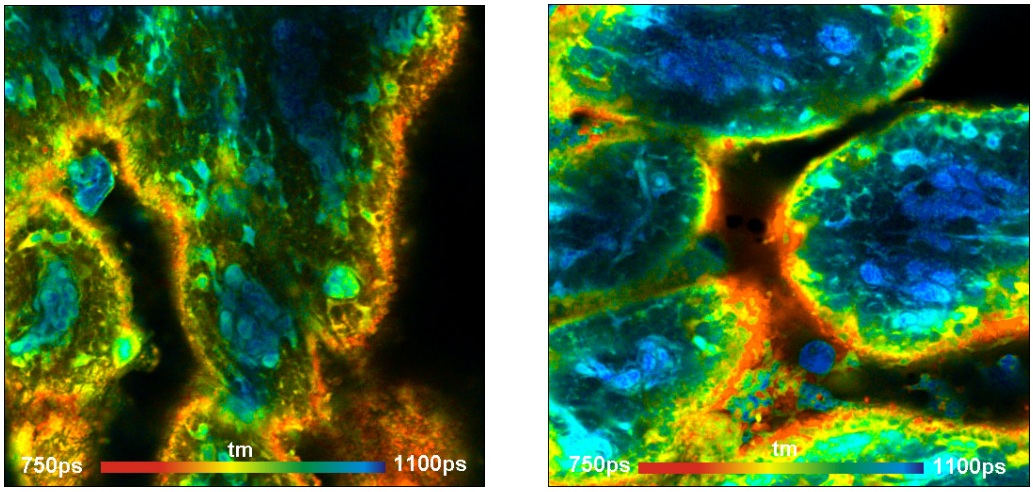


Fig. 41: Pig skin samples stained with 3,3'-diethylthiatricarbocyanine. Zeiss LSM 780 NLO system, one-photon excitation by Ti:Sa laser at 780nm, confocal detection at 800nm to 900nm

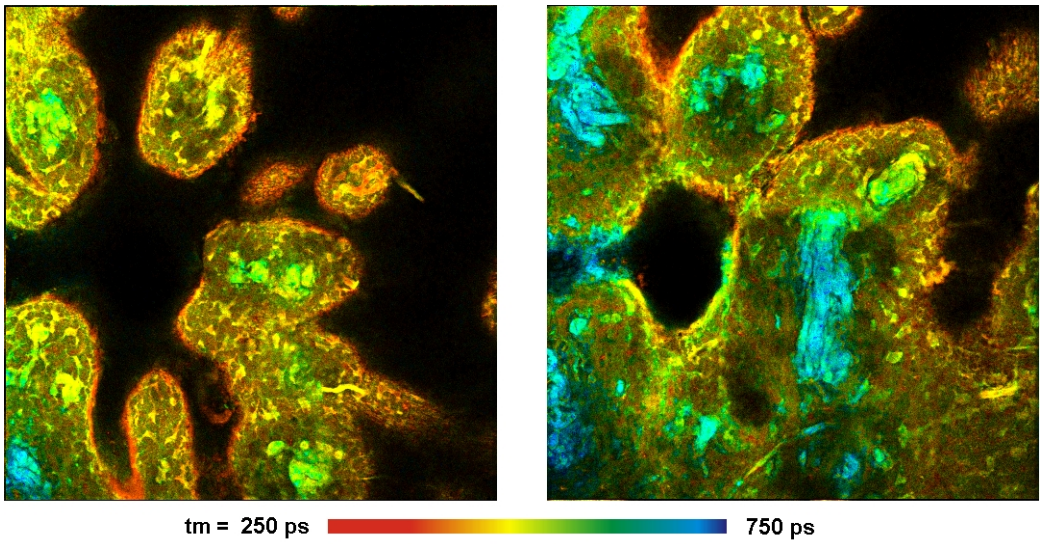


Fig. 42: Pig skin stained with Indocyanin Green. Zeiss LSM 780 OPO system, two-photon excitation at 1200 nm, non-descanned detection, 780 to 850 nm. Depth from top of tissue 10 µm (left) and 40 µm (right).

FLIM / PLIM: Simultaneous Fluorescence and Phosphorescence Lifetime Imaging

Phosphorescence and fluorescence lifetime images are recorded simultaneously by bh's proprietary FLIM/PLIM technique. The technique is based on modulating a ps diode laser synchronously with the pixel clock of the scanner. FLIM is recorded during the 'On' time, PLIM during the 'Off' time of the laser [9, 34, 35, 44]. The SPCM software delivers separate images for the fluorescence and the phosphorescence which are then analysed with SPCImage FLIM/PLIM analysis software.

Currently, there is increasing interest in PLIM for background-free recording and, especially, for oxygen sensing. In these applications, the bh technique delivers a far better sensitivity than PLIM techniques based on single-pulse excitation. The real advantage of the bh FLIM/PLIM technique is, however, that FLIM and PLIM are obtained *simultaneously*. It is thus possible to record metabolic information via FLIM of the NADH and FAD fluorescence, and simultaneously map the oxygen concentration via PLIM [41, 42]. The number of publications in this area is literally exploding, please see FLIM/PLIM chapters in [1], [2] or [34]. An example is shown in Fig. 43.

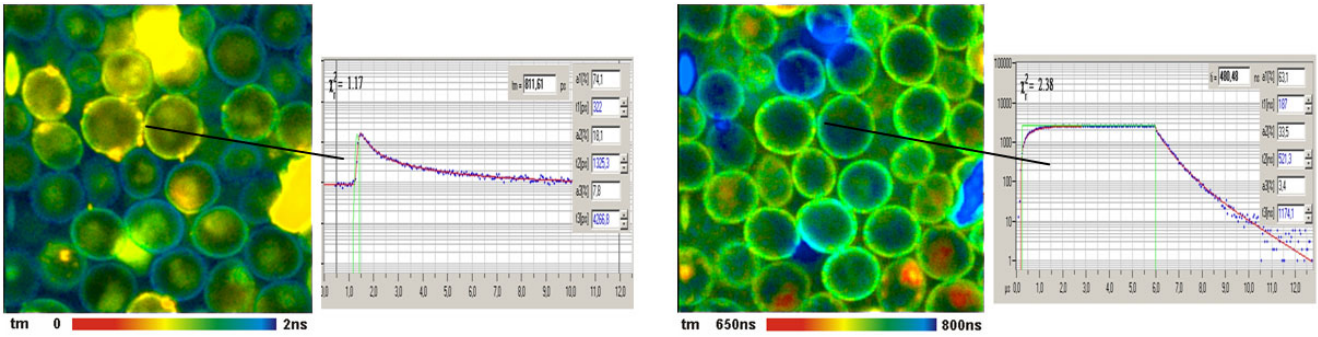


Fig. 43: Yeast cells stained with (2,2'-bipyridyl) dichlororuthenium (II) hexahydrate. FLIM and PLIM image, decay curves in selected spots.

FLIM of Macroscopic Objects

With the bh DCS-120 MACRO version objects as large as 15 mm can be scanned [2]. Image obtained with the DCS-120 MACRO is shown in Fig. 44 and Fig. 45.

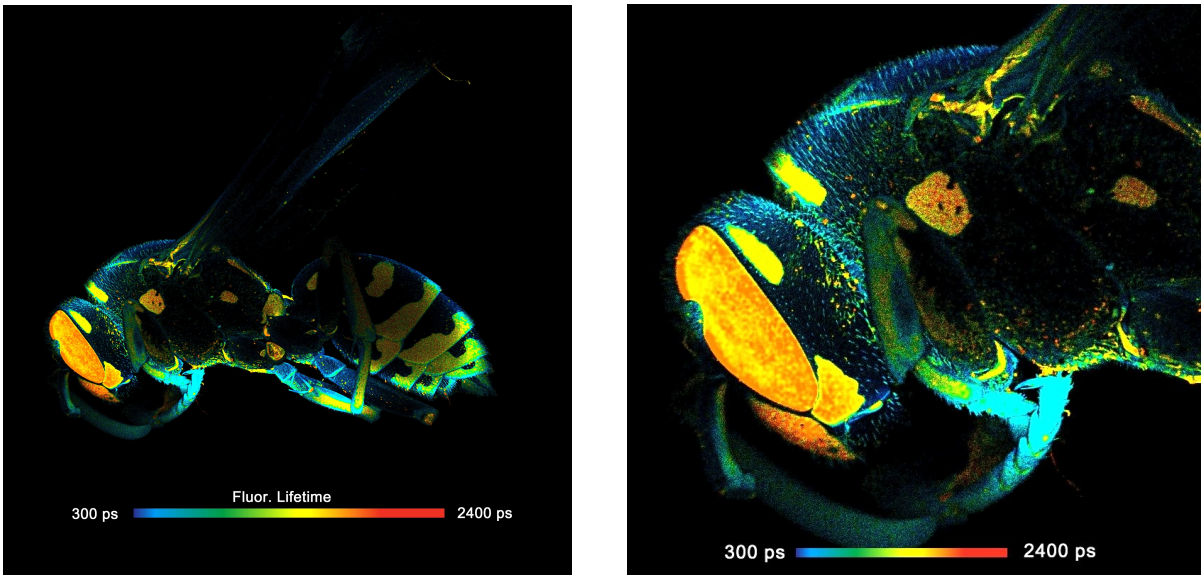


Fig. 44: FLIM of a macroscopic object. Resolution 2048 x2048 pixels, 256 time channels. Left: Original image. Right: digital zoon into recorded FLIM image, showing the excellent resolution of the data.

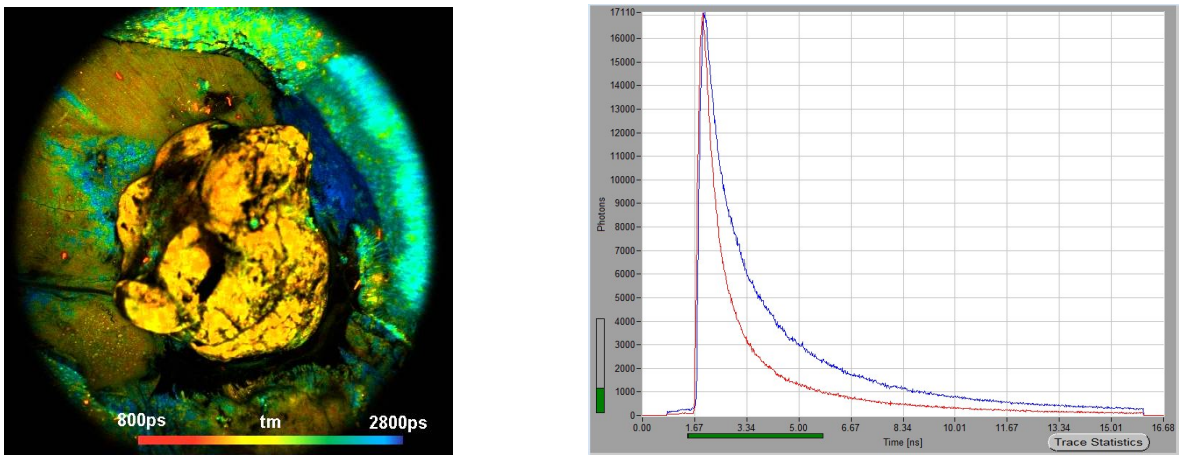


Fig. 45: Tumor in a live mouse. NADH FLIM image (left) and decay curves inside and outside tumor (right).

Scanning of Well Plates

With an optional motor stage, both the DCS-120 and the DCS MACRO system can be used to scan well plates. An example is shown in Fig. 46.

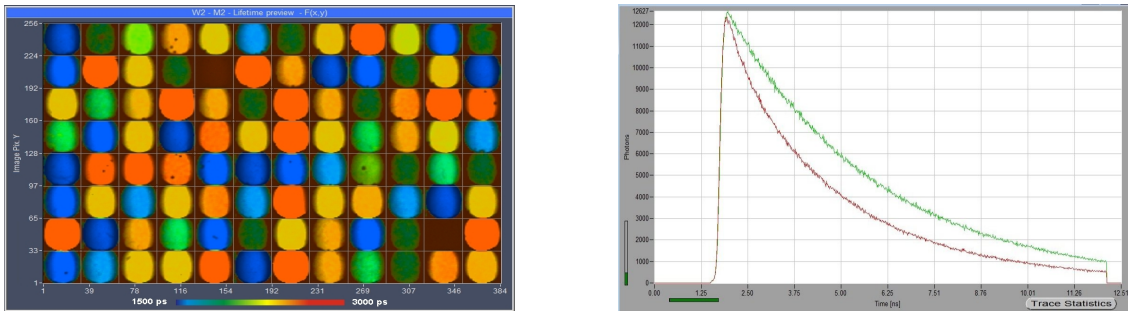


Fig. 46: Well plate scanned with DCS-120 MACRO. Lifetime image and decay functions in wells 4 and 5, lower row.

STED FLIM

TCSPC FLIM can be combined with STED [34]. The combination of a STED microscope of Abberior Instruments (Göttingen, Germany) with the bh Simple-Tau 150/154 TCSPC FLIM system records FLIM data at a spatial resolution of better than 40 nm. The image format can be as large as 2048 x 2048 pixels, with 256 time channels per pixel. An image area of 40 x 40 micrometers can thus be covered with 20 nm pixel size, fully satisfying the Nyquist criterion. With smaller numbers of time channels even larger pixel numbers are possible. The system especially benefits from Windows 64 bit technology used both in the Abberior and in the bh data acquisition software, from the combined processing power of two parallel system computers, and the high data throughput of up to four parallel TCSPC FLIM channels. The system achieves peak count rates in excess of 5 MHz per FLIM channel, resulting in unprecedented signal-to-noise ratio and short acquisition time.

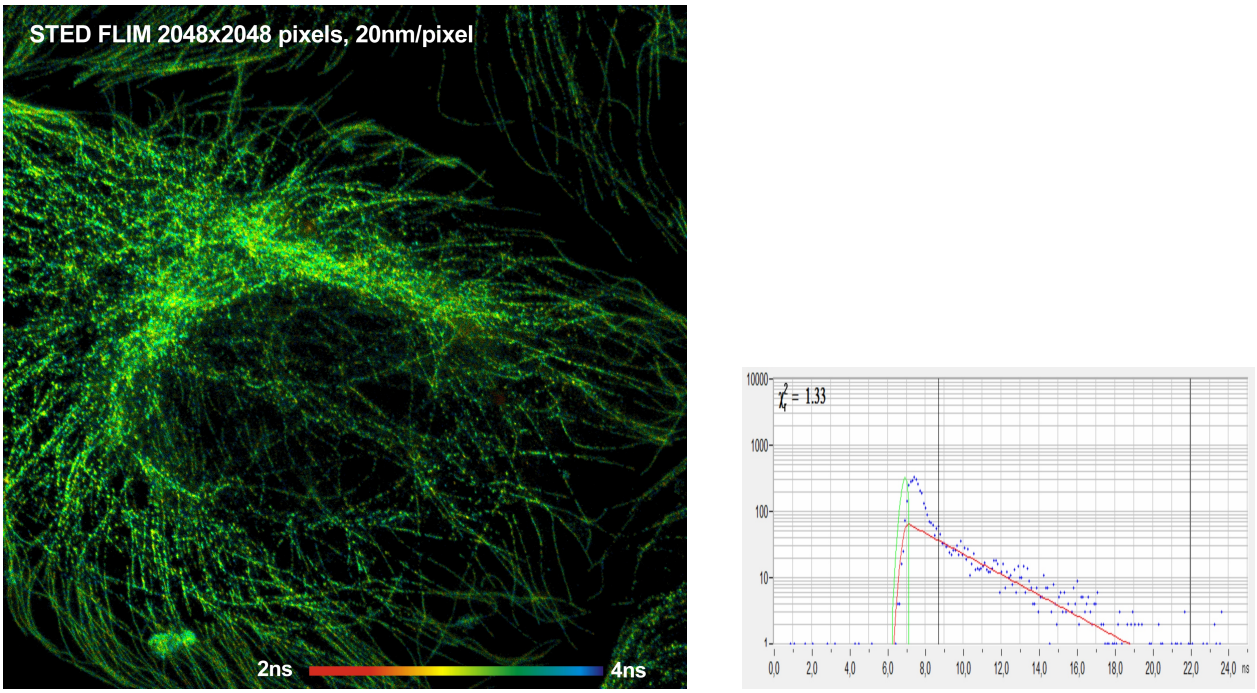


Fig. 47: STED FLIM with Abberior Instruments STED microscope. 2048 x 2048 pixels. Single cell, stained with tubulin-binding dye, recorded at a resolution of 20 nm per pixel. Decay curve in selected pixel shown on the right. The initial peak is undepleted fluorescence. It is gated off in the intensity data of image shown on the left.

Clinical FLIM

Clinical FLIM applications use the fact that pathological processes induce changes in the molecular environment or in the conformation of endogenous fluorophores. These, in turn, cause detectable changes in the fluorescence decay profiles. bh FLIM has been introduced into clinical instruments for ophthalmology and dermatology. Developments for other applications are in progress. Please see [33] or [34] for an overview and for technical details. The first clinical instruments are on the market. FLIM images recorded with the FLIO Fluorescence Lifetime Ophthalmoscope of Heidelberg Engineering and with the MPT Flex multiphoton skin tomography system of Jenlab are shown in Fig. 48.

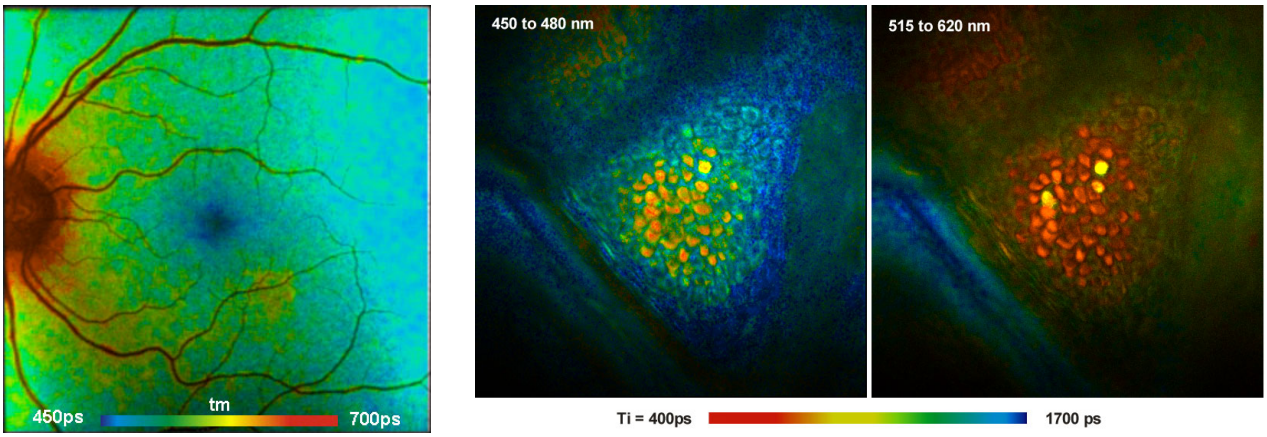


Fig. 48: Left: FLIM of a human retina, recorded in vivo with Heidelberg Engineering FLIO ophthalmoscope. Right: Multiphoton FLIM of human skin, recorded in vivo with Jenlab MPT FLEX multiphoton tomography system.

FCS

The bh GaAsP hybrid detectors of the bh FLIM systems deliver highly efficient FCS [1, 29, 34]. Because the detectors are free of afterpulsing there is no afterpulsing peak in the autocorrelation data. Thus, accurate diffusion times and molecule parameters are obtained from a single detector. Compared to cross-correlation of split signals, correlation of single-detector signals yields a four-fold increase in correlation efficiency. The result is a substantial improvement in the SNR of FCS recordings [29, 34]. FCS is be obtained both with confocal systems and with multiphoton NDD systems. Gated FCS is obtained by hardware gating the photon times within the TCSPC modules, FCCS by cross-correlating the signals of two TCSPC channels.

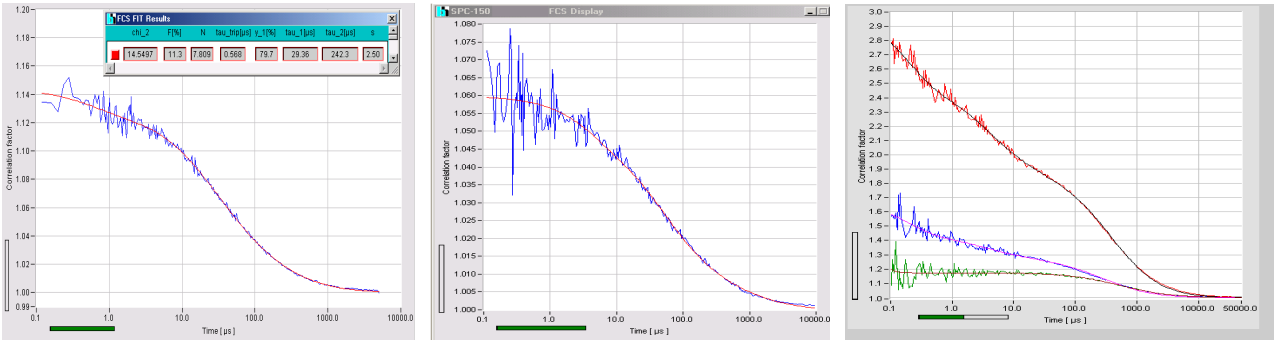
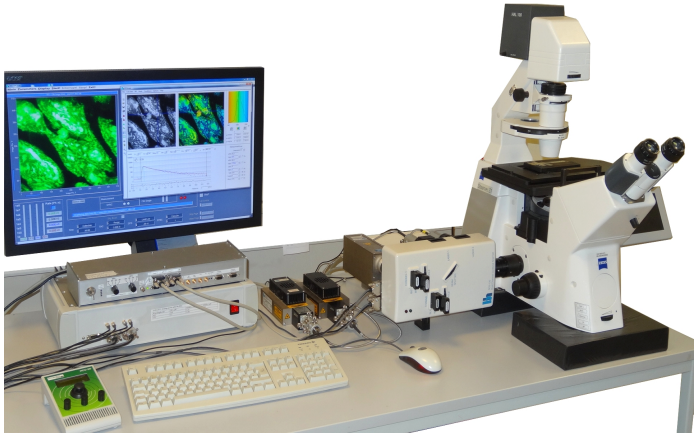
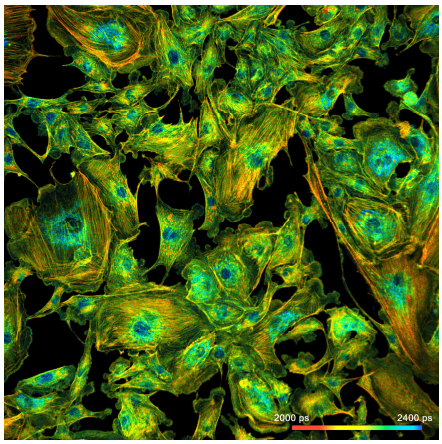


Fig. 49: FCS with bh TCSPC FLIM systems, GaAsP hybrid detectors. Left to right: Confocal FCS with ps diode laser, two-photon NDD FCS, cross correlation of photons recorded in different detection channels.

bh FLIM Systems for Various Microscopes

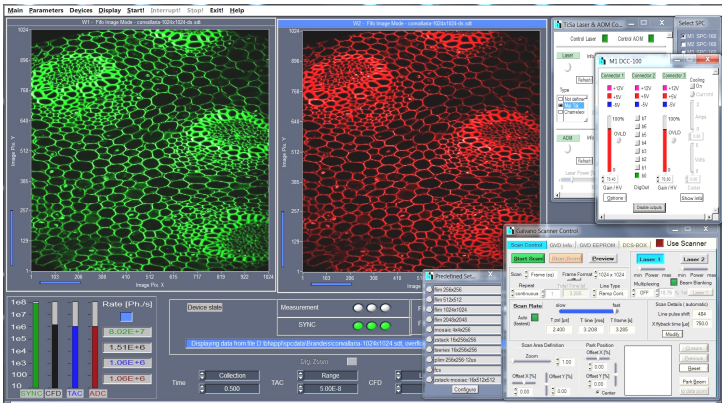
DCS-120 Confocal Scanning FLIM Systems

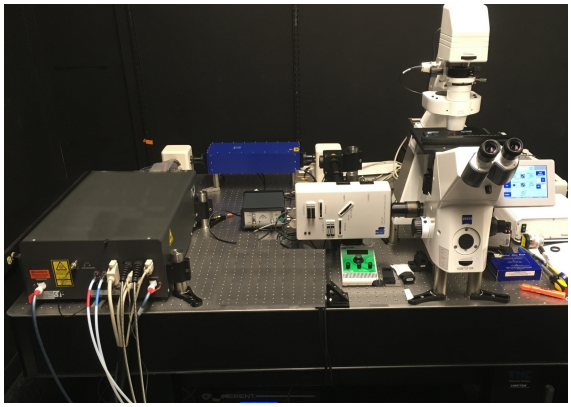




FLIM at image size up to 2048 x 2048 pixels
Complete Confocal Laser Scanning FLIM microscopes
FLIM upgrade for existing conventional microscopes
Scanning by fast galvanometer mirrors
Two fully parallel confocal detection channels
One or two BDL-SMC or BDL-SMN picosecond diode lasers
Laser wavelengths 375, 405, 440, 473, 488, 510, 640, 685, 785 nm
Wideband (WB) version, compatible with tuneable lasers
Channel separation by dichroic or polarising beamsplitters
Individually selectable pinholes, individually selectable filters
bh HPM-100-40 GaAsP hybrid detectors
Optional HPM-100-06 detectors for ultra-high time resolution
GaAs hybrid detectors for NIR range
Optional 16-channel multi-wavelength GaAsP detector module
Z-stack FLIM acquisition with Zeiss Axio Observer Z1
Simultaneous FLIM / PLIM
Optional motor stage, control integrated in instrument software
Spatial and temporal mosaic FLIM
Ultra-fast recording of time series
Metabolic FLIM Capability
Fluorescence lifetime-transient scanning (FLITS)
Version with FASTAC FLIM available
Please see [2] for details

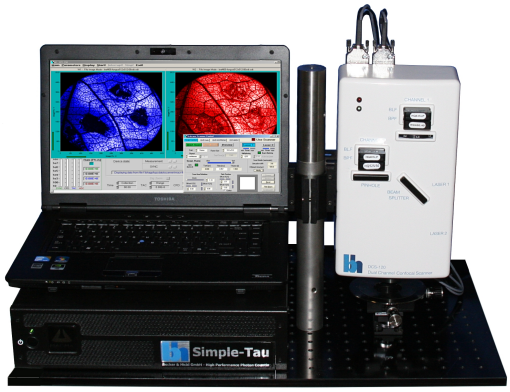
DCS-120 MP Multiphoton FLIM Systems

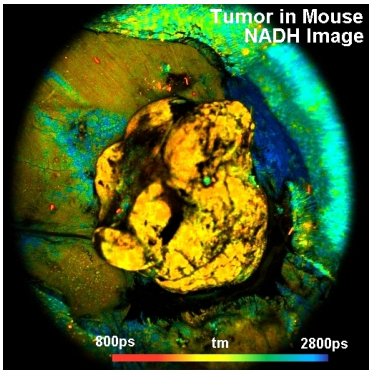


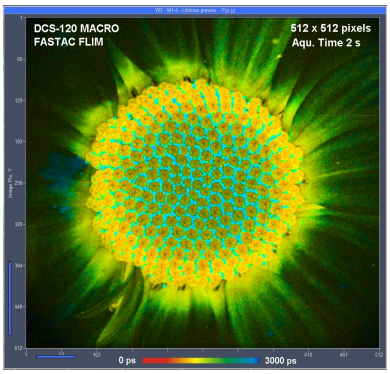


Excitation by fs Ti:Sa laser or fs fibre laser
Laser intensity and wavelength control integrated in SPCM data acquisition software
PLIM laser modulation by DCS-120 scan controller and AOM, control functions integrated in instrument software
Clear Images from deep tissue layers
Excellent spatial and temporal resolution
Full field of view of microscope lens scanned
Images up to 2048 x 2048 pixels
Two non-descanned detection channels,
Two optional confocal channels
bh HPM-100-40 GaAsP hybrid detectors
Optional HPM-100-06 detectors for ultra-high time resolution
Optional 16-channel multi-wavelength GaAsP detector module
Z-stack FLIM acquisition with Zeiss Axio Observer Z1
Simultaneous FLIM / PLIM
Optional motor stage, control integrated in instrument software
Spatial and temporal mosaic FLIM
Ultra-fast recording of time series
Metabolic-FLIM capability
Fluorescence lifetime-transient scanning (FLITS)
FASTAC FLIM system available
Please see [2] for details

DCS-120 Macro System



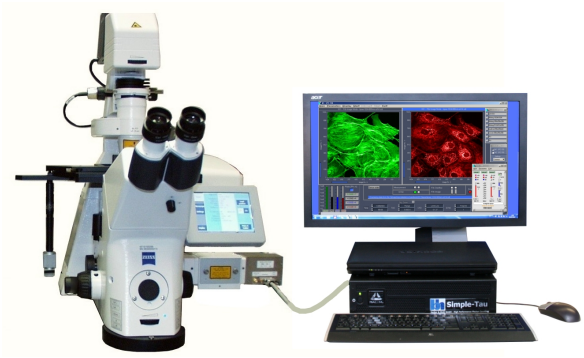


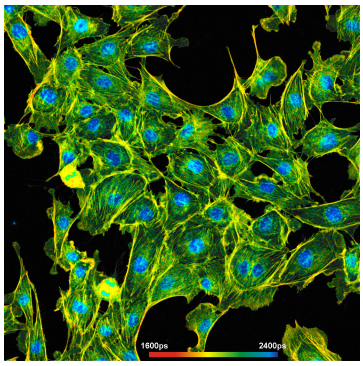


FLIM of macroscopic objects
Scan field up to 15 mm diameter
FLIM with up to 2048 x 2048 pixels
Scanning by fast galvanometer mirrors
Two fully confocal detection channels
One or two BDL-SMC or BDL-SMN picosecond diode lasers
Laser wavelengths 375, 405, 440, 473, 488, 510, 640, 685, 785 nm
Tuneable excitation by super-continuum laser with AOTF
One or two confocal detection channels, parallel acquisition
Channel separation by dichroic or polarising beamsplitters
Individually selectable pinholes, individually selectable filters
bh HPM-100-40 GaAsP hybrid detectors
Optional HPM-100-06 detectors for ultra-high time resolution
GaAs hybrid detectors for NIR range
16-channel multi-wavelength GaAsP detector module
Optional motor stage, control integrated in instrument software
Simultaneous fluorescence and phosphorescence lifetime imaging (PLIM)
Metabolic FLIM capability
Spatial and temporal mosaic FLIM
Ultra-fast recording of time series
Fluorescence lifetime-transient scanning (FLITS)
Wideband (WB) version, compatible with tuneable lasers
FASTAC FLIM system available
Please see [2] for details

FLIM Systems for Zeiss LSM 710 / 780 / 880 / 980 Microscopes

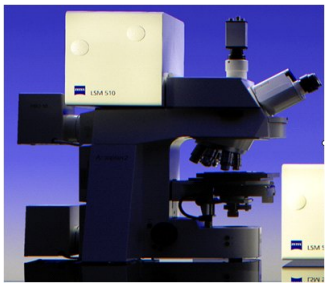
LSM 710 / 780 / 880 / 980 NLO, LSM 7MP Multiphoton Microscopes
LSM 710, LSM 780, LSM 880, LSM 980 Confocal Microscopes

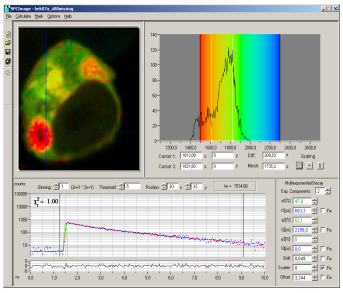




FLIM with up to 2048 x 2048 pixels
Multiphoton FLIM, PLIM, multispectral FLIM, FCS
Confocal FLIM, PLIM, multispectral FLIM, FCS
FLIM with bh HPM hybrid detectors or Zeiss BIG-2 detectors
Fast preview mode, both for intensity and lifetime
Mosaic FLIM
Z Stack FLIM
Fast Time-series FLIM
Acquisition by 1, 2, 3 or 4 parallel TCSPC FLIM channels
Detection by bh HPM-100-40 GaAsP hybrid detectors or Zeiss BIG 2 detector
Optional HPM-100-06 detectors for ultra-high time resolution
Simultaneous fluorescence and phosphorescence lifetime imaging (PLIM)
Fluorescence lifetime-transient scanning (FLITS)
Spatial and temporal mosaic FLIM
Ultrafast time-series recording by temporal mosaic FLIM function
Confocal NIR FLIM up to 900 nm detection wavelength
Two-Photon OPO FLIM up to 900nm detection wavelength
FASTAC FLIM system available
Please see [1] for details

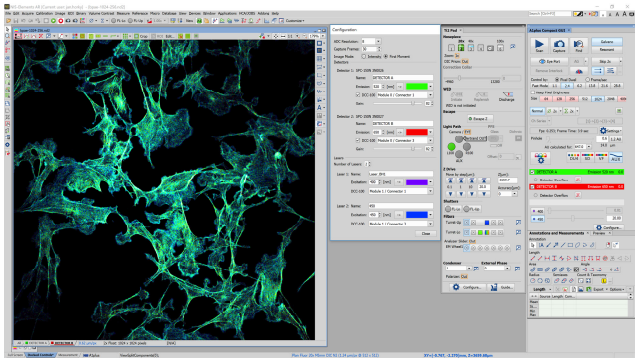
Still available: FLIM Systems for Zeiss LSM 510 NLO Multiphoton Microscopes

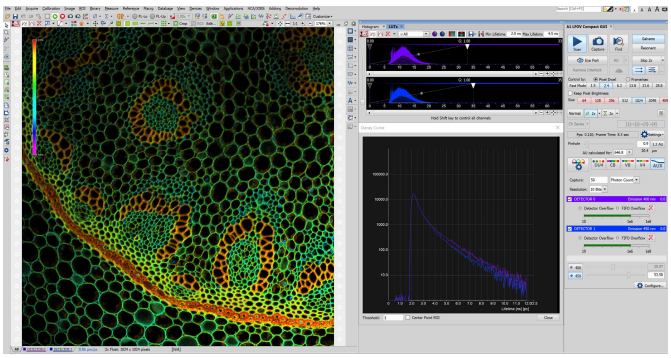




FLIM with up to 2048 x 2048 pixels
Multiphoton excitation with non-descanned detection
Single-wavelength and Dual-wavelength NDD FLIM
Multi-spectral NDD FLIM
Fast preview mode
Mosaic FLIM
Z Stack FLIM
Fast time-series FLIM
HPM-100-40 hybrid detectors
One or two parallel SPC-150 TCSPC channels
Portable to LSM 710, 780, 880 microscopes

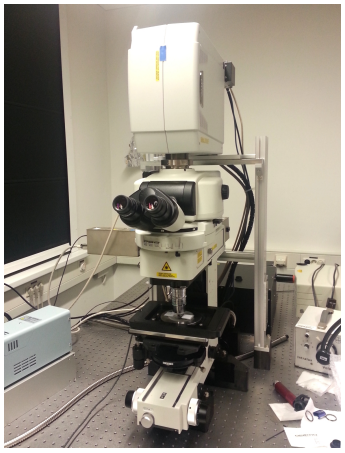
Software-Integrated FLIM for Nikon A1+ Confocals

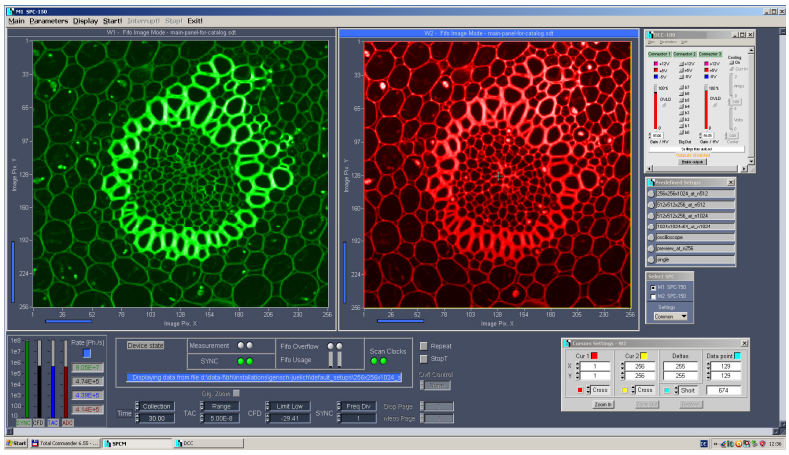




Integrated in Nikon's NIS-Elements Instrument Software
Excitation by bh BDS-SM ps diode lasers
Detection by bh HPM-100-40 GaAsP hybrid detectors
Two fully parallel SPC-150N TCSPC FLIM channels
Data analysis by bh SPCImage
Fast acquisition, high optical resolution, high efficiency, high time resolution
Please see [18] for details

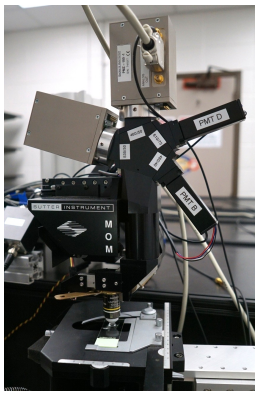
Non-descanned FLIM Systems for Nikon A1 MP Multiphoton Microscopes

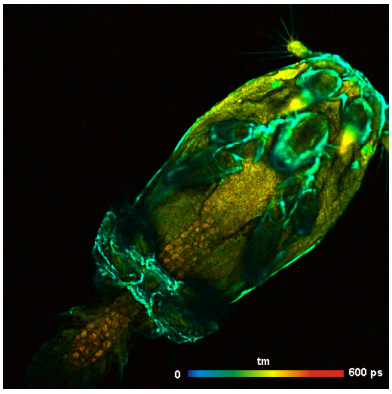




64-bit megapixel FLIM technology
One FLIM channel or two parallel FLIM channels
High-efficiency PMH-100 hybrid detectors
Non-descanned detection for deep-tissue imaging
Multi-spectral FLIM with 16-channel GaAsP detector
ROI and Zoom functions of A1 available
Works at any scan rate
Megapixel FLIM
Fluorescence lifetime-transient scanning (FLITS)
Ultra-fast time series by temporal mosaic FLIM
FASTAC FLIM system available

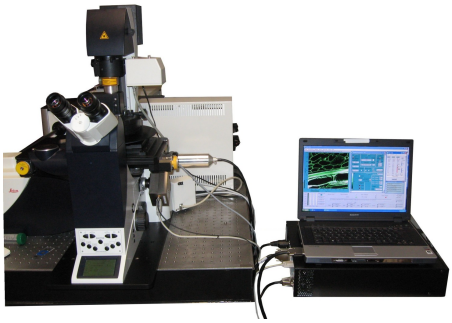
FLIM Systems for Sutter Instrument MOM Microscopes

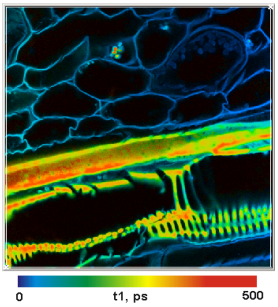




Up to four parallel FLIM channels
Multiphoton excitation by Ti:Sa laser
Non-descanned detection for deep-tissue imaging
Overload protection of FLIM detectors
Up to 1024 x 1024 pixels, 1024 time channels
High efficiency
Fast acquisition
SPCM Online FLIM function available
Simultaneous FLIM / PLIM
FASTAC FLIM system available
Please see [12] for details.

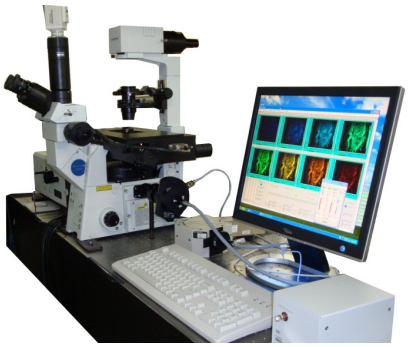
Non-Descanned FLIM Systems for Leica SP5 MP, SP8 MP Microscopes

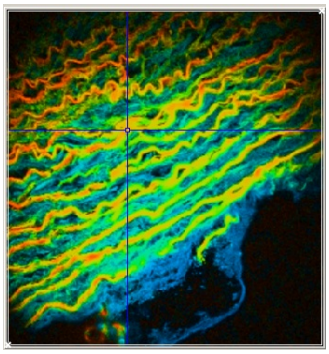




Non-descanned detection via Leica RLD port
1 detector coupled directly to RLD port
2 detectors via external beamsplitter
Simple-Tau 150 or 152 TCSPC systems
Acquisition in 1 or 2 parallel TCSPC FLIM channels
bh HPM-100-40 GaAsP hybrid detectors or Leica HYD detectors
Optional HPM-100-20 ultra-fast hybrid detectors
Multi-spectral FLIM with 16-channel GaAsP detector
Works at any scan rate of SP microscope
No nonlinearity by Leica sinusoidal scan
Fast acquisition, fast preview mode
Megapixel FLIM, 2048 x 2048 pixels
Fluorescence lifetime-transient scanning (FLITS) and temporal mosaic FLIM available
Ultra-fast time series by temporal mosaic FLIM
Simultaneous FLIM / PLIM
FASTAC FLIM system available
Please see [6] for details.

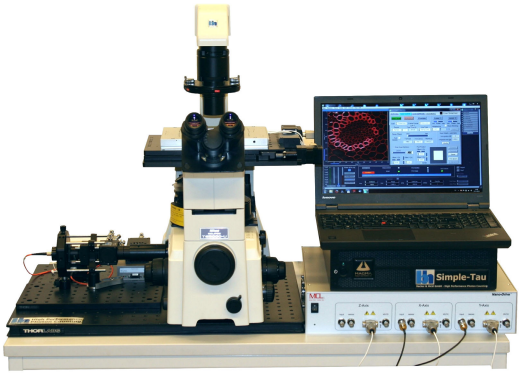
Non-descanned FLIM Systems for Olympus Multiphoton Microscopes

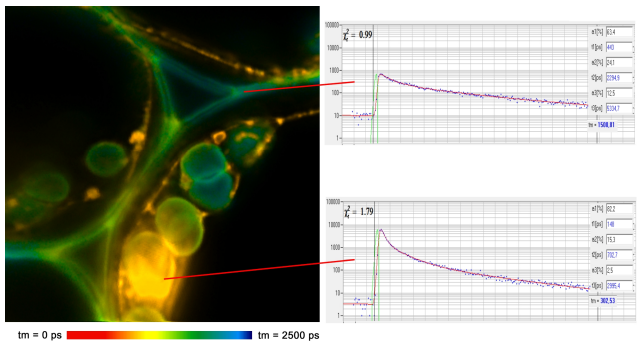




Multiphoton FV systems with inverted microscopes
High efficiency by non-descanned FLIM detection
Deep-tissue imaging capability
HPM-100-40 GaAsP hybrid detectors
Optional HPM-100-06 hybrid detectors for ultra-high time resolution
Optional 16-channel multi-spectral GaAsP detector
Full overload protection of FLIM detectors
ROI and Zoom functions of available
Works at any scan rate
Fluorescence lifetime-transient scanning (FLITS) and temporal mosaic FLIM available
FASTAC FLIM system available

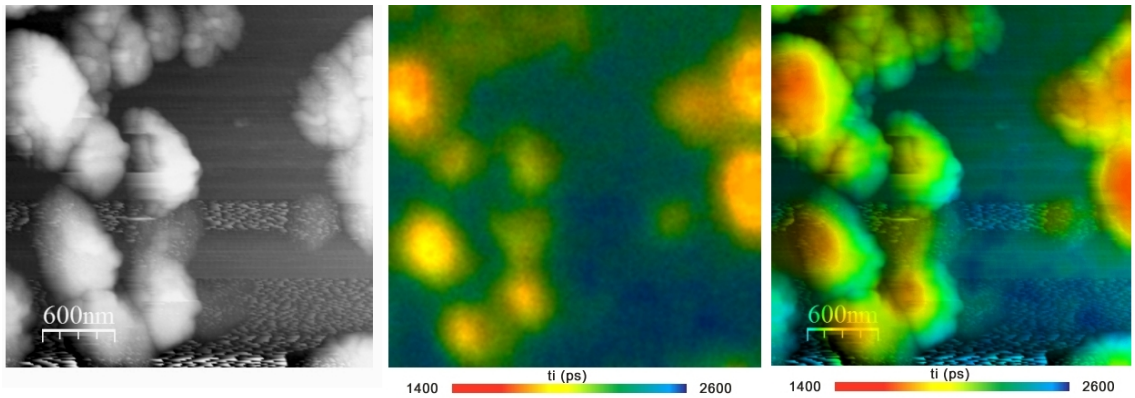
PZ-FLIM-110 Stage-Scanning FLIM System





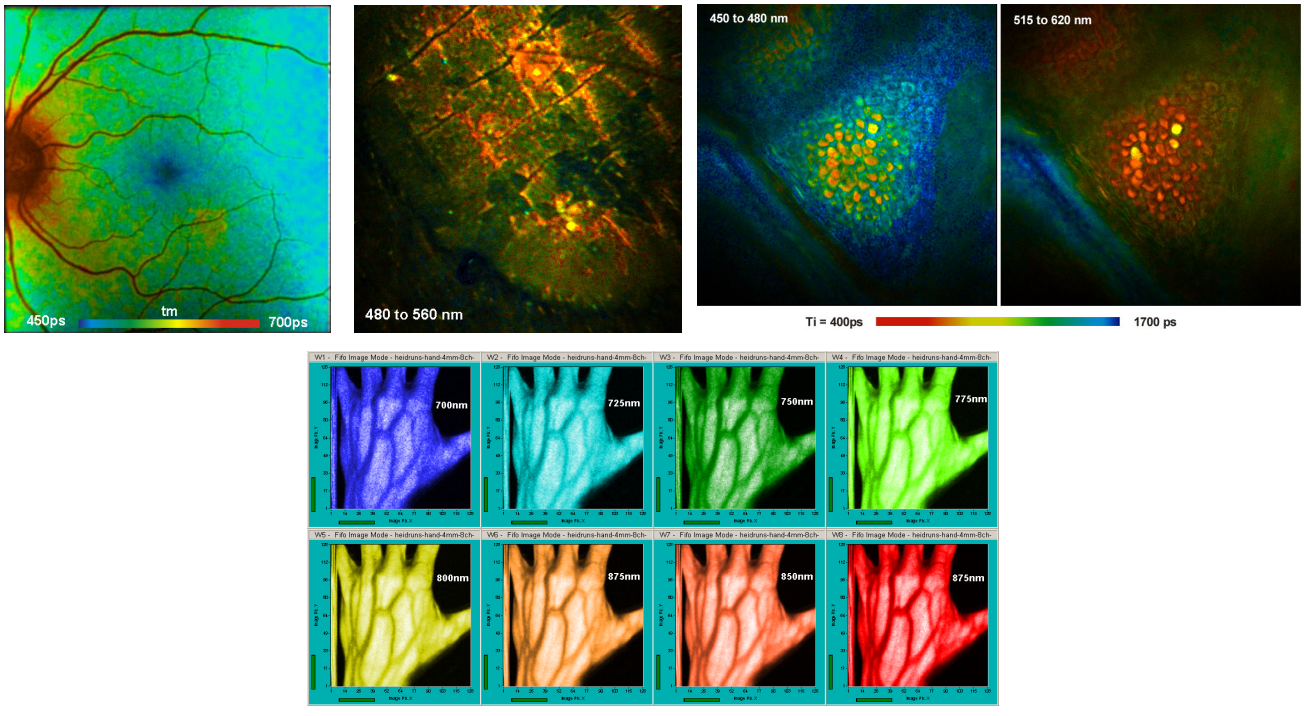
Sample scanning by piezo scan stage
Excitation by BDL or BDS series ps diode lasers
Confocal detection
HPM-100-40 GaAsP hybrid detector
Optional HPM-100-06 hybrid detector for ultra-high time resolution
Optional PML-SPEC GaAsP multi-spectral detector
Excellent contrast and resolution
Fully controlled by bh SPCM TCSPC/FLIM data acquisition software
Compact electronics, integrated in bh Simple Tau system
Megapixel FLIM technology - images up to 2048 x 2048 pixels
Lateral (x-y) an vertical (z) scanning
Simultaneous FLIM / PLIM
Please see [34] for details.

FLIM for NSOM Systems



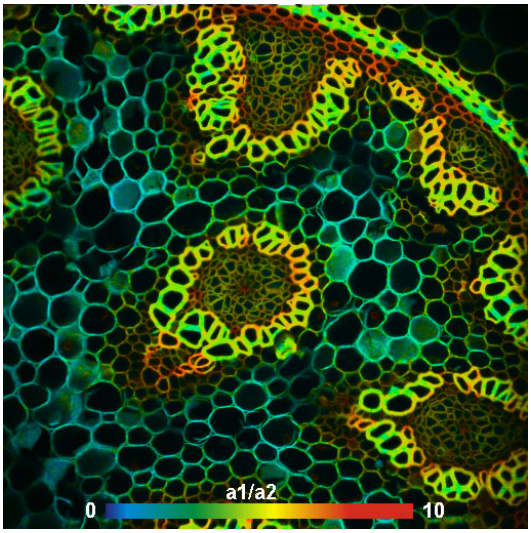
For NSOM systems of Nanonics, MD-NDT and others
Combines atomic-force and fluorescence lifetime information
High sensitivity by HPM-100-40 GaAsP hybrid detectors
Optional HPM-100-06 detectors for ultra-high time resolution
Fluorescence and phosphorescence lifetime imaging
Single-point transient-lifetime recording
Please see bh TCSPC Handbook [34]or contact bh.

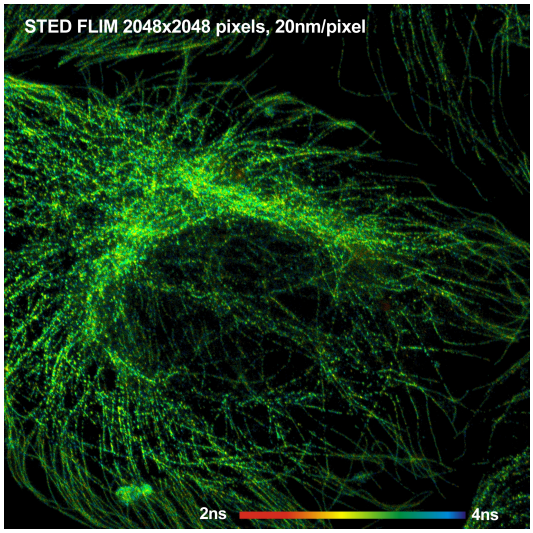
FLIM Systems for Clinical Imaging



FLIM systems for ophthalmology
FLIM systems for dermatology
FLIM systems for tissue imaging
FLIM through endoscopes
Time-resolved NIRS and fNIRS Imaging
Online FLIM at rates of up to 10 images per second
Please see bh TCSPC Handbook [34] or contact bh

FLIM for other Scanning Systems





Left: FLIM recorded with Lucid Vivascope, ultra-fast polygon scanner. Right: STED FLIM recorded with STED microscope of Abberior Systems, Goettingen

bh FLIM systems can be configured for almost any conceivable laser scanning system. They work with galvanometer scanners, polygon scanners, resonance scanners, and motor-driven and piezo-driven scan stages.
Please see bh TCSPC Handbook [34] or contact bh.

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For more references on the bh FLIM technique plaese see W. Becker, The bh TCSPC Handbook, available on www.becker-hickl.com.

Specifications

General Principle

Lifetime measurement	time-domain
Excitation	high-frequency pulsed lasers
Buildup of lifetime images	Single-photon detection by multi-dimensional TCSPC [34] Builds up distribution of photons over photon arrival time after laser pulses, scan coordinates, time from laser modulation, time from start of experiment.
Multi-wavelength FLIM	uses wavelength of photons as additional coordinate of photon distribution
Excitation wavelength multiplexing	uses laser number as additional coordinate of photon distribution
Scan rate	works at any scan rate
Buildup of fluorescence correlation data	correlation of absolute photon times [34]
General operation modes	FLIM, two spectral or polarisation channels Multi-wavelength FLIM Time-series FLIM, microscope-controlled time series Z-Stack FLIM Mosaic FLIM, x,y, z, temporal Excitation-wavelength multiplexed FLIM FLITS (fluorescence lifetime-transient scanning) PLIM (phosphorescence lifetime imaging) simultaneous with FLIM FCS, cross FCS, gated FCS, PCH Single-point fluorescence decay recording

Data recording hardware, please see [34] for details

TCSPC System	bh Simple Tau 152 TCSPC system, inside PC or extension box coupled to laptop			
Number of parallel TCSPC / FLIM channels	up to 4			
Number of detector (routing) channels in FLIM modes	16 for each FLIM channel			
Principle	Advanced TAC/ADC principle			
Electrical time resolution, IRF width, SPC-150, SPC-160	2.3 ps rms / 6.8 ps fwhm			
Minimum time channel width, SPC-150, SPC-160	813 fs			
Electrical time resolution, IRF width, SPC-150NX	1.6 ps rms / 3.5 ps fwhm			
Minimum time channel width, SPC-150NX	405 fs			
Timing stability over 30 minutes	typ. better than 5ps			
Dead time	100 ns			
Saturated count rate	10 MHz per channel			
Dual-time-base operation	via micro times from TAC and via macro time clock			
Source of macro time clock	internal 40MHz clock or from laser			
Input from detector	constant-fraction discriminator			
Reference (SYNC) input	constant-fraction discriminator			
Synchronisation with scanning	via frame clock, line clock and pixel clock pulses			
Scan rate	any scan rate			
Synchronisation with laser multiplexing	via routing function			
Recording of multi-wavelength data	simultaneous in 16 channels, via routing function			
Experiment trigger function	TTL, used for Z stack FLIM and microscope-controlled time series			
Basic acquisition principles	on-board-buildup of photon distributions buildup of photon distributions in computer memory generation of parameter-tagged single-photon data online auto or cross correlation and PCH			
Operation modes	f(t), oscilloscope, f(txy), f(t,T), f(t) continuous flow FIFO (correlation / FCS / MCS) mode Scan Sync In imaging, Scan Sync In with continuous flow FIFO imaging, with MCS imaging, mosaic imaging, time-series imaging Multi-detector operation, laser multiplexing operation cycle and repeat function, autosave function			
Max. Image size, pixels (SPCM 64 bit software)	4096x4096	2048x2048	512x512	256x256
No of time channels, see [34]	64	256	1024	4096

Data Acquisition Software, please see [34] for details

Operating system	Windows 7 or Windows 10, 64 bit
Loading of system configuration	single click in predefined setup panel
Start / stop of measurement	by operator or by timer, starts with start of scan, stops with end of frame

Online calculation and display, FLIM, PLIM	in intervals of Display Time, min. 1 second
Online calculation and display, FCS, PCH	in intervals of Display Time, min. 1 second
Number of images displayed simultaneously	max 8
Number of curves (Decay, FCS, PCH, Multiscaler)	8 in one curve window
Cycle, repeat, autosave functions	user-defined, used for for time-series recording, Z stack FLIM, microscope-controlled time series
Saving of measurement data	User command or autosave function Optional saving of parameter-tagged single-photon data
Link to SPCImage data analysis	automatically after end of measurement or by user command

Data Analysis: bh SPCImage, integrated in bh TCSPC package, see [1, 2] or [34]

Data types processed	FLIM, PLIM, MW FLIM, time-series, Z stacks, single curves
Procedure	iterative convolution or first-moment calculation
IRF	synthetic IRF or measured IRF
Model functions	single, double, triple exponential decay single, double, triple exponential incomplete decay models shifted-component model
Parameters displayed	amplitude- or intensity-weighted average of component lifetimes ratios of lifetimes or amplitudes, FRET efficiency fractional intensities of components or ratios of fractional intensities parameter distributions
Parameter histograms, one-dimensional	Pixel frequency over any decay parameter or ratio of decay parameters
Parameter histograms, two-dimensional	Pixel frequency over two decay parameters, Phasor plot

Excitation Sources, One-Photon Excitation, please see [1] for details

Picosecond Diode Lasers	bh BDS-SM or BDL-SMC lasers
Number of lasers	max 4
Number of lasers simultaneously operated (multiplexed)	2
Available wavelengths	375nm, 405nm, 445nm, 473nm, 488nm, 515nm, 640nm, 685nm, 785nm
Mode of operation	picosecond pulses or CW
Pulse width, typical	30 to 100 ps
Pulse frequency	selectable, 20MHz, 50MHz, 80MHz
Power in picosecond mode	0.4mW to 1mW injected into fibre. Depends on wavelength version.
Power in CW mode	20 to 40mW injected into fibre. Depends on wavelength version.
Other Vis-Range Lasers	
Visible and UV range	any ps pulsed laser of 20 to 80 MHz repetition rate
Coupling requirements	Point Source-Kineflex compatible fibre adapter
Wavelength	any wavelength from 370nm to 785nm
Synchronisation / Modulation of Lasers	
Laser Multiplexing	Diode lasers, pixel by pixel, line by line, frame by frame DCS-120: Integrated in scan controller Other microscopes: multiplexing requires bh DDG-210 card
Interleaved excitation	Sync of diode laser to diode laser
Laser Modulation for PLIM	Integrated in DCS system, otherwise requires bh DDG-210 card

Excitation Sources, Multi-Photon Excitation

Femtosecond NIR Lasers	any femtosecond Ti:Sa laser, Ti:Sa-pumped OPO, or fs fibre laser
Wavelength	700nm to 1000nm
Repetition rate	40 to 80 MHz
Laser Modulation for PLIM	integrated in DCS MP systems, otherwise requires bh DDG-210 card and AOM

Detectors

GaAsP Hybrid Detectors (standard)	bh HPM-100-40 hybrid detector
Spectral Range	300 to 710nm
Peak quantum efficiency	40 to 50%
IRF width, FWHM	110 to 130 ps
Detector area	3mm
Background count rate, thermal	300 to 2000 counts per second
Background from afterpulsing	not detectable

Afterpulsing peak in FCS	not detectable
Power supply and overload shutdown	via DCC-100 controller of TCSPC system
Ultra-Fast Hybrid Detectors	bh HPM-100-06 hybrid detector
Spectral Range	300 to 600 nm
Peak quantum efficiency	20 %
IRF width (with Ti:Sa laser of fs fibre laser)	<20 ps
Detector area	3mm
Background count rate, thermal	300 to 1000 counts per second
Background from afterpulsing	not detectable
Power supply and overload shutdown	via DCC-100 controller of TCSPC system
Hybrid Detectors for NIR (optional)	bh HPM-100-50 hybrid detector
Spectral Range	400 to 900nm
Peak quantum efficiency	12 to 15%
IRF width with bh diode laser	120 to 180 ps
Detector area	3mm
Background count rate, thermal	1000 to 8000 counts per second
Background from afterpulsing	not detectable
Power supply and overload shutdown	via DCC-100 controller of TCSPC system
Multi-Wavelength FLIM Detector (optional)	bh MW GaAsP FLIM assembly
Spectral range	380 to 750nm
Number of wavelength channels	16
Spectral width of wavelength channels	12.5 nm
IRF width, FWHM	250 ps
Power supply and overload shutdown	via DCC-100 controller of TCSPC system

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